ACADEMIC FREEDOM AND UNIVERSITY STANDARDS

IN contrast to the protests, so far unsuccessful, against the infringement of university autonomy in South Africa by the Union Government's Extension of University Education Act, which is universally recognized throughout the academic world as striking against the fundamental conception of a university, a recent *Bulletin of the Committee on Science and Freedom* (No. 14; February 1960) records a successful outcome to a storm of protest from academic and public opinion in Queensland. This was against a Government measure which attempted to establish an officially sponsored appeal board through which the Government could control university staff appointments. This outcome is perhaps the more encouraging in that it demonstrated the existence of an unexpected number of allies in a community where generally graduates—and also scientists, as the Murray Committee's report on Australian universities has shown—are still a small minority and one not held in high enough repute. It should be noted, however, that freedom of platform and Press is far more of a reality in Australia than in South Africa, and this, as Prof. T. K. Ewer shows in the article referred to above, is an important factor when a vital liberty is threatened.

Prof. Ewer describes how the threat arose when the Government of Queensland introduced in 1957 the University of Queensland Acts Amendment Act, 1957. Since the eight universities in Australia, except those of Sydney and Melbourne, are almost wholly dependent on Government funds, the legislation under which they operate seeks to assure strong Government representation on the governing bodies. This Act incorporated a number of amendments which were acceptable to the Staff Association and to the Senate, although a request of the former for three representatives on the Senate was unacceptable to that body, which nevertheless supported the principle of direct representation of staff. This, however, was limited to one member, and so far, in spite of the large expansion of university staff in the past decade and strong representations by the University Staff Association to the Minister of Education, this number has not been increased to the suggested three. In fact, the lack of sympathy shown by Government representatives on the Senate to such matters as an acceptable superannuation scheme, staff housing and the maintenance of parity in salary scale with the Universities of Sydney and Melbourne emphasize both the importance of an informed and alert public opinion and of the comments of the Murray Committee. The expenditure by the Australian Government in the next twelve years of £A20 million recommended by the Murray Committee will not by itself suffice to remove the shortage of scientists and technologists in Australia in academic or other employment, unless their work is appreciated by the community and paid for in accordance with its value.

The real threat described by Prof. Ewer, however, arises from Sections 25 and 31 in the Act, which stated the principle of allowing appeals, to be made to a specially constituted appeal board, against academic and non-academic appointments made by the University and also against disciplinary action. This provision was not to apply to the offices of vice-chancellor or registrar, but the composition of the appeal board raised the main criticism and opposition within and without the University. This was to consist of three members: one appointed by the Government, who was to be chairman; one appointed by the Senate; and one appointed by the Staff Association. These provisions caused the most vigorous protest throughout Australia; it is not quite clear how the blunder was made, but the processes of democracy saved the day.

Without going into the full details, it may be noted that a public meeting of some 2,400 people in the City Hall, Brisbane, passed with acclamation a resolution stating that the provision of an appeal board is "a direct threat to academic freedom and to academic standards of the university; departs from the tradition and procedures of universities within the British Commonwealth; is a great disservice to the interests of the people of Queensland". Pointed reference was made at the meeting to the threat which political interference with academic appointments offered to recruitment, particularly for professorships and senior posts, where reliance is still largely on overseas applicants. The defeat of the Government in a general election, however, and return of a new Government to power was followed by repeal of the appeal board provisions on December 17, 1958, and the new University of Queensland Acts Amendment Act, omitting the appeal board provisions, was proclaimed on August 11, 1958.

This unexpected attack on the University's autonomy has been followed by much discussion among the University staff, and the Professorial Board reconsidered the methods hitherto used for making appointments at all levels. Proposals were framed to ensure that every applicant for a new post on the University staff would be interviewed by the selection committee; the existing system of committees and referees is much the same as that employed in other British universities. The vital lesson of the episode, however, seems to be the importance of public understanding and the existence of effective democratic institutions.

It is the absence of these in South Africa that has been responsible for the passing of the unfortunate Extension of University Education Act and for its operation. Already eight members of the staff of

the University College of Fort Hare have been dismissed by the South African Government under the Act, without reason being given; and it seems clear that opposition to government policy will now be regarded as grounds for dismissal and that the members of staff thus dismissed, who include the registrar, five professors, the librarian and a lecturer, are the first victims of the political control of university institutions for non-white peoples. Speaking at the meeting of the Tunis study group of the Committee on Science and Freedom on "Freedom and Responsibility: the Role of the Scholar in Society" in April 1959, Dr. E. G. Malherbe maintained that the idea of a university completely controlled by the State was repugnant to South African men who had learned to value the traditional British system of freedom and autonomy. While, however, the Act would affect adversely the recruitment of staff of good quality and thus tend to debase South Africa's academic currency, its most serious effect would be the exclusion of non-white students from existing universities. Unless the Government instituted a very elaborate system of bursaries, it was difficult to see how the millions of non-whites who are living outside the projected Bantustans, and are completely integrated into the economy of the white people, would have any chance to obtain a university education. Apart from considerations of autonomy and academic freedom, the issue would become economic. South Africa, for a civilized country, has a very low national income per head, and it is difficult to see how she could aspire to multiply university institutions merely in the interests of preserving the ethnic identity of peoples who have evolved no written language and whose culture is riddled with witchcraft and magic.

Dr. Malherbe was confident that, apart from this principle of exclusion in the new legislation, the academic freedom and autonomy of the existing universities of South Africa continue to stand as high as in any country in the world; but with these dismissals at Fort Hare before us it may well be doubted how long this will last. The free society anywhere depends for its strength upon the exercise of civic responsibility by its members, and though the academic community can play a major part by the manifestation of courage and strength of conviction in maintaining the vigorous growth of the ideal and reality of freedom, it cannot continue indefinitely to function as a community unless those qualities command sufficient respect among the public at large to ensure maintenance of the essential conditions of academic freedom of teaching, investigation and appointment. The conference at which Dr. Malherbe's paper was given provided encouraging evidence of the extent to which the idea of academic freedom has taken root, and at the present time it is particularly interesting to note this in the papers by Prof. B. A. Fletcher on the university and society in Central Africa, by Prof. E. Njoku on the relation between university and society in Nigeria, and by Dr. H. A. Oluwasanmi on the scholar and society in Nigeria.

Prof. Fletcher touched briefly on the way in which the University College of Rhodesia and Nyasaland, at Salisbury, is seeking to serve its society in respect of teaching, research and intellectual guidance, emphasizing that in industry, politics and religion it should leave leadership to others. It can both serve society and nourish itself by research and collaborative inquiry in the major fields of science, the social sciences and education, though these are not the only avenues. It was clear enough that Prof. Fletcher's vision of the service a university can render its society can only be realized in a university free from control by Government or any external agency, and free to determine for itself what it shall teach and whom it shall admit, freedoms which in general, as Dr. J. W. Cook observed in an introductory paper on university traditions with particular reference to the British Commonwealth, can only be safeguarded if the academic community has a large measure of control over appointments to its own body.

Prof. Njoku was optimistic that, given proper cultivation, academic freedom should root strongly in Nigeria, and Dr. Oluwasanmi, observing that the Western university tradition is still new in Nigeria, pointed out that, though Ibadan University College derives the bulk of its finances from the State, it has succeeded in maintaining its academic autonomy. It is essential, however, he said, that such university institutions in multi-racial societies should increasingly draw their inspiration from the environment in which they live and function if they are to accept the additional, if non-traditional, function of creating conditions from which a national, as distinct from tribal or racial, identity can be forged. This means that such institutions must also give more attention to those unexplored regions of knowledge that emphasize the common elements of the diverse cultures of the territories in which they function.

There was no tendency at this meeting to ignore the dangers which political and other conditions offer to the realization of these ideals: they were explicitly faced by both Prof. Njoku and Dr. Oluwasanmi, and equally by Dr. Cook, who put his finger on some of the obstacles which the surging tide of nationalism, for example, can raise. While it is easy to sympathize with the desire to foster the use of national languages, it can be an important factor in preventing the attainment of satisfactory standards in a university as well as hinder the free exchange of information and thought with the Western world, particularly in science, where a substantial proportion of original literature is published in English. A university has a duty to guard against any action which might weaken it in its pursuit of learning and the search for knowledge in the less advanced countries, no less than in the advanced and highly industrialized countries.

Dr. Cook said he is convinced that the policy adopted in 1945 in developing these new university institutions in the Commonwealth countries had been right. High academic standards have been established from the start, staffs of high calibre appointed, and significant and important contributions to new

knowledge, often related to the needs and opportunities of the regions, have been made. There has also been a small but steadily increasing flow of graduates who will have a profound influence on the development of their nations.

All this will be of increasing importance as more territories move to independence and increasing efforts are made to solve the problems of government in multi-racial territories. More university institutions will probably be required, and Dr. Cook wisely suggests that these need not be conceived on precisely the same lines as the existing institutions. There is room for experiment, and perhaps especially in ways that have closer regard to the financial resources of newly independent countries. What matters most is that in giving careful attention to the traditional cultures of the countries and to their expanding needs, high academic standards should be maintained and the university traditions which have stood the test of time should be scrupulously observed. For that reason alone, universities in the rapidly developing countries should be the more careful not to become embroiled in political controversy, and to ensure that their legitimate studies of political, economic and social questions should be undertaken in no partisan spirit. Nor may it be too much to hope that in the measure in which these new universities meet the needs of the independent territories, and especially of the multi-racial communities, they will demonstrate the futility of the South African Government's present university policy, and perhaps even cause its reversal before South African academic traditions are destroyed and irreparable damage done to its universities.

ANTIBIOTICS FROM A NOVEL ANGLE

Antibiotics

Their Chemistry and Non-Medical Uses. Edited by Dr. Herbert S. Goldberg. Pp. x+608. (Princeton, N.J.: D. Van Nostrand Company, Inc., London: D. Van Nostrand Company, Ltd., 1959.) 112*s*. 6*d*.

IN a foreword to this very welcome book, Dr. Paul R. Burkholder, the discoverer of chloromycetin, says: "For some time past, all of us have been certain about one aspect of antibiotic science and that is the great need for a new kind of book which would summarize the remarkable advances that have taken place recently" and the reviewer, who is in full agreement, would like to add "particularly in their non-medical uses". The book is the work of six American authors, all of whom are well known in antibiotic science. It consists of an introduction and six chapters, each of which is a complete monograph in itself. An unusual and very useful feature of the book is the large number of references—some two thousand in all—occupying a total of 261 pages and amounting to about 43 per cent of the book. The references given in the introduction (216 in number) and in the opening chapter on the chemistry of antibiotics (566) give the full title of the paper referred to in the text together with the usual details. In the remaining five chapters, however, very informative abstracts of the contents of the papers are also given.

The introduction deals with the history of antibiotics, their economic import, natural occurrence, effects and mode of action. The opening chapter (84 pages) admirably succeeds in summarizing the chemistry of the penicillins, streptomycin and related antibiotics, chloromycetin, the tetracycline group, the macrolides, novobiocin, polyene and polypeptide antibiotics.

The growth stimulation and general effects on the nutrition of domestic animals and birds is discussed in the following chapter (59 pages; 545 references). The magnitude and importance of this aspect of antibiotics may be judged from the fact that in 1954 more than 20 per cent (value 25 million dollars) of all antibiotics produced in the United States were used in animal feed supplements.

Botanists, and particularly plant pathologists and physiologists, will find the article on "The Influence of Antibiotics on Plants and Plant Disease Control" (78 pages; 376 references) a full and authoritative account of the subject. It is certainly surprising to learn that at least thirty-nine different antibiotics have been studied in the control of plant bacterial and fungal pathogens.

The texts of the remaining three chapters are relatively short. Chapter 5 (22 pages; 283 references, which unfortunately are not numbered) deals with antibiotics in food preservation, that is, in canned foods, fresh foods including meats, poultry, fish, fruit and vegetables, dairy products and yeast fermentations. Laboratory workers in microbiology will particularly welcome chapter 6 (18 pages; 96 references) on the uses of antibiotics in the isolation in pure culture and cultivation of micro-organisms from the complex mixtures in which they normally occur, for example, bacteria, fungi including plant pathogens, protozoa, viruses and rickettsiae. The book closes with an authoritative article (22 pages; 75 references) on the public health significance of non-medical uses of antibiotics, particularly in foods for human consumption. We may however take some comfort from the author's opinion that "one must conclude that the seriousness of the public health hazards of antibiotic residues in food has been overstated".

I believe that this book will completely satisfy the need expressed earlier for a new kind of book on antibiotic science. HAROLD RAISTRICK

BIOCHEMICAL PRACTICE

Biochemical Preparations

Vol. 6. Edited by Carl S. Vestling. Pp. ix+105. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.) 42*s*. net.

Methods of Biochemical Analysis

Vol. 7. Edited by Prof. David Glick. Pp. ix+353. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959.) 72*s*.

THE latest volume in the valuable series of "Biochemical Preparations" contains detailed descriptions of carefully checked methods for the preparation of the following: crystalline animal cytochrome *c*, deoxyribonucleic acid, 2,3-diphosphoglyceric acid, L-α-glycerophosphorylcholine, 3-

hydroxyanthranilic acid, β-hydroxy-β-methylglutaric acid, insulin, lanosterol, leucine amino-peptidase, α-methylserine and *bis* (hydroxymethyl) glycine, crystalline horse oxyhæmoglobin, old yellow enzyme, crystalline papain and benzoyl-L-argininamide, crystalline muscle phosphorylases *a* and *b*, phosphoserine, rat liver ribonucleic acid, ribulose diphosphate, and DL-tryptophan-7a-C^{14}. A few minor criticisms are worthy of mention. The excellent practice of including structural formulæ was apparently overlooked in the description of the preparation of lanosterol. It is regrettable that anachronisms such as "Twenty-one and two-tenths grams of L-arginine hydrochloride . . ." (p. 64) and "Two and six-tenths kilograms of the washed and dried yeast . . ." (p. 55) are preferred to the more usual form. Grammatical errors, such as "The enzyme is extracted from dried brewer's bottom yeast, impurities removed by precipitation with basic lead acetate, and the enzyme further purified by fractionation . . ." (p. 54), and "The combined eluates are dialyzed and the adsorption procedure repeated . . ." (p. 57), were surprising.

The editorial board clearly makes strenuous efforts to replace older methods in earlier volumes by better methods, as illustrated by the inclusion of improved preparations of muscle phosphorylase and cytochrome *c*. There must be an interval before this is possible, however, and it is suggested that an appendix in each volume might give references to recent descriptions in the original literature of new methods of preparation of compounds, which have formed the subject-matter of earlier volumes of this series. One example will suffice; the preparation of leucine aminopeptidase has been simplified (Folk, Gladner, and Viswanatha, *Biochim. Biophys. Acta*, **36**, 256; 1959) since this volume was published, and, although not checked by independent workers, reference to the method would be helpful in succeeding volumes. There is a valuable list of compounds of biochemical interest which have appeared in "Organic Syntheses" up to Vol. 38.

The volumes of "Methods of Biochemical Analysis" have rightly assumed a position of authority in biochemical literature. The latest volume deals with eight topics. Various modifications of the technique of immunoelectrophoresis are described in detail by P. Grabar, who is a pioneer in this field. This will please many workers, for much of the original literature appears in recondite journals. Two articles of considerable value to toxicologists describe the analysis of basic nitrogenous compounds (A. S. Curry) and the determination of ethyl alcohol in blood and tissues (F. Lundquist). The latter would provide the Minister of Transport with some interesting home-work. Workers in the lipid field are catered for by two chapters entitled "The Determination of Inositol, Ethanolamine, and Serine in Lipides" (J. M. McKibbin) and "The Assay of Lipoprotein Lipase *in vivo* and *in vitro* (E. D. Korn). L. B. Jaques and H. J. Bell have given a detailed description of the bewildering variety of methods for the extraction and determination of heparin. This article, however, contains the astonishing statement that "Barium due to its high molecular weight will dilute and hence reduce the values". The rather short account of the determination of creatinine and related guanidinium compounds (J. F. Van Pilsum) could have included a more critical discussion of the numerous colorimetric methods for arginine determination. Several recent methods (Satake and Luck, *Bull. Soc. Chim. Biol.*, **40**, 1743; 1958: Cerriotti and Spandrio, *Biochem. J.*, **66**, 603; 1957: Janus, *Nature*, **177**, 529; 1956) are not mentioned. The recently developed technique for spectrophotometric study of translucent materials, which employs an opal glass in the light beam, is described by Shibata. Theoretical principles are considered, and suitable attachments for commercial spectrophotometers are described.

D. T. Elmore

THE WAY TO THE STARS

Advances in Astronautical Sciences

Vol. 1: Proceedings of the Third Annual Meeting of the American Astronautical Society, December 1956. Pp. vi+177. Vol. 2: Proceedings of the Fourth Annual Meeting, December 1957. Pp. viii+456. Vol. 3: Proceedings of the Western Regional Meeting, August 1958. Pp. viii+547. Vol. 4: Proceedings of the Fifth Annual Meeting, November 1958. Pp. ii+460. (New York: Plenum Press, Inc.; London: Chapman and Hall, Ltd., 1957, 1958 and 1959.) 80*s*. net each volume.

ASTRONAUTICS, rejoicing in respectability and a far-flung dependence on numerous aspects of science and technology, finds itself admirably suited to congresses and conventions. The records of the proceedings tend to be impressive, expensive and distinctly daunting to the general reader, however great his specialist knowledge in one or two related fields.

The present volumes are no exception: they record the proceedings of four separate meetings of the American Astronautical Society held in December 1956 (Vol. 1), December 1957 (Vol. 2), August 1958 (Vol. 3) and November 1958 (Vol. 4). Each volume costs 8 dollars which, by some curious transformation process, becomes £4 in Great Britain. Taken together, they comprise 143 papers with some abstracts and supplementary lectures, covering 1,640 pages and weighing 8 lb. Vol. 1, which is less than half the size of any of the others but costs just as much, begins with a short paper by C. S. Draper on "Education in the Astronautical Sciences". He suggests a 4–6-year curriculum likely to overwhelm all but the most able and enthusiastic student. Many of the following papers are speculative rather than specific, and the volume ends with much discussion about the twin or clock paradox and relativity.

The remaining three volumes are all post-*Sputnik* 1 and reflect the enormous impetus that the launching of the world's first artificial satellite gave to American astronautics. An appreciable proportion of the latter volumes consists of sober theoretical analyses of the motions of space vehicles, communication and navigation problems, reports of full-scale and laboratory experiments and the development of ideas based on verified facts or current techniques. In all these matters the exposition is generally excellent, and we are—to use a somewhat inappropriate simile—on solid ground. Some of the remaining papers are futuristic and here the reviewers find themselves rather at a loss. In most fields of engineering, development is achieved by making an accurate estimate of the worthwhile magnitude of the next step forward, on technical and economic grounds. One can easily think of examples in aviation where the steps have been either too conservative or too

bold. When reading about ideas in astronautics, the practical applications of which are often exceedingly complex but which advance very rapidly, it is difficult to judge just how far ahead the author may be thinking. It certainly seems that some of the brains devoted to contemplating future developments have taken their thoughts well beyond the next few feasible technological steps: in the absence of a yardstick, it becomes difficult to distinguish between far-ranging speculation or suggestion and something very close to science fiction. One can only marvel at the opulence of an industry capable of supporting gentlemen who devote their thoughts to "Assembly of a Multi-Manned Satellite" particularly on reading sentences such as: "Imagine then a space-suited worker chasing tools and materials on different orbits". Of course, such papers are intensely interesting, particularly when written in concise English, as is this particular example. Papers using turgid prose are fortunately in the minority.

One is therefore caused to question the object of all this activity, and is gratified to find that Dryden's contribution to the introduction of Vol. 4 is entitled "Space Exploration and Human Welfare". It is probably unfair to expect a concisely stated objective: the paper lists a few minor benefits to television and meteorology, says quite bluntly that we do not know the ultimate role of space vehicles in transportation and makes reference to the desire for increasing our knowledge of the universe for peaceful purposes. It seems quite possible that something is left unsaid.

The binding and paper of these volumes are excellent: unfortunately the reproduction of the contents is very variable in quality. The first three volumes consist of reproductions of typescript and hand-written equations. The diagrams reproduce well, but most of the photographs are very unsatisfactory. Volume 4 reverses this situation. The print and photographs are generally good but the diagrams, while clear, are reproduced on far too small a scale.

These volumes contain much valuable information and would be useful additions to a generously endowed library.

F. G. Irving
J. L. Stollery

PLANT PHYSIOLOGY IN THE FIELD

Plants and Environment

A Textbook of Plant Autecology. By Prof. R. F. Daubenmire. Second edition. Pp. xi+422. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 56*s*. net.

THIS is a revised edition of a book originally published in 1947. The format and the heavy glossy paper are typically North American. The text is enriched by many photographs and diagrams; some of the photographs appear to have suffered in reproduction.

As Prof. Daubenmire's introductory definition of autecology in his preface shows, the sub-title of this book is misleading to British ecologists, most of whom would define autecology as the study of the ecology of a single species. This book, on the contrary, deals with the environmental factors which affect plants. Seven factors—soil, water, temperature, light, atmospheric, biotic, and fire—are given a chapter each, and two further chapters deal with "The Environmental Complex" and with "Ecologic Adaptation and Evolution". Much useful information on recent work is included, particularly in the fields of plant physiology and genecology. Pursuing his interpretation of autecology, the author discusses the environmental factors as they affect particular plants, but the plant examples are chosen at random, and nowhere is there an integrated study of a single species such as a British ecologist would expect from "A Text-book of Plant Autecology". Nor is there any reference to the plant as part of a natural community, nor any analysis of different types of vegetation, nor any reference to the distribution of plants and historical ecology; presumably all these subjects fall within the author's definition of synecology and are deliberately excluded, but it is surely impossible to study the autecology of a species without at least considering these aspects. The approach to ecology presented in this book is therefore that of the experimentalist rather than that of the naturalist; indeed, "Plant Physiology in the Field" would be an apter title than "A Text-book of Plant Autecology".

Within these limits, the book will be of interest to plant geographers and should prove a useful reference book to students of botany. It provides data on many subjects which are relatively unfamiliar in Britain, such as frost action on soils, anatomical details of frost damage and sun scorch, anatomical effects on trees of the adverse habitats encountered at the upper timber-line of natural forest, and the ecological effects of widespread fires. Sections in the chapters on water factors and light factors provide a very useful bridge between plant physiology and ecology, and there is much miscellaneous but useful information in the chapters on atmospheric factors and biotic factors.

W. Tutin

NON-INDIGENOUS ANIMALS OF GREAT BRITAIN

The Ark in Our Midst

The Story of the Introduced Animals of Britain—Birds, Beasts, Reptiles, Amphibians, Fishes. By R. S. R. Fitter. Pp. 320+16 plates. (London: William Collins, Sons and Co., Ltd., 1959.) 18*s*. net.

THIS is an ambitious book for in it the author sets out to cover a wide field, to survey the non-indigenous animals of the British Isles, that is the mammals, birds, reptiles and amphibians, which owe their inclusion in our fauna to the agency of man, creatures so diverse as the edible frog, the pheasant and the grey squirrel; and he does not restrict himself to those that have been successfully naturalized but includes species that have failed to establish themselves, pointing out that they are not the less interesting because they lack the necessary adaptability. Mr. Fitter tells us that the failures have been many and in excess of the successes, though the latter have often been spectacular with far-reaching results, for example, the manner in which the rabbit, the rat and the grey squirrel have colonized Great Britain.

Although we may be sure that man was the agent concerned in the arrival here of a number of creatures, although we may be positive they did not get here 'under their own steam', it is by no means easy to ascertain in each case how the deed was done, still less when it was done, even when it must have been well within the historic period.

It has long been the custom to give great credit to the Romans as importers of animals, much of which credit they undoubtedly deserve; yet evidence is singularly lacking as to what beasts, birds, fishes and so on they really did bring across the English Channel and naturalize in Britain. It is said that they introduced the pheasant; yet the bird bones found in Romano-British settlements have all been identified by experts as belonging to the domestic fowl. But we do find evidence that the pheasant was in Britain by Norman times. In a charter of 1098, the Bishop of Rochester assigned to that City among other things sixteen pheasants from certain Kentish manors, and in 1100 the Abbot of Malmesbury was given a royal licence to kill hares and pheasants, which latter we may presume were as naturalized and wild as the hares.

Mr. Fitter's industry in the collection of facts regarding the arrival in Britain of this variety of creatures has been immense. His lengthy volume is packed with information, the facts being clearly and concisely related, resulting in a most useful book that will be valuable for reference for many years to come.

Frances Pitt

THE ZOOGEOGRAPHY OF WESTERN NORTH AMERICA

Zoogeography

Edited by Carl L. Hubbs. (A Symposium presented on August 26–27, 1957, at the Stanford University Joint Meeting of the American Institute of Biological Sciences and the Pacific Division of the American Association for the Advancement of Science, and a Symposium presented on December 28, 1957, at the Indianapolis Meeting of the American Association for the Advancement of Science.) Pp. x+509. (Publication No. 51.) (Washington, D.C.: American Association for the Advancement of Science; London: Bailey Bros. and Swinfen, Ltd., 1958.) 108*s*.

THIS volume contains seventeen full articles dealing with the wide variety of topics to be expected in two symposia on zoogeography. It does not deal much with the subject as a whole, and the title is really misleading, since most of the articles are concerned only or mainly with the western part of North America. The topics covered include the present and past distribution of land areas, habitats and climate (Tertiary and Pleistocene), physiological tolerance of vertebrates as bearing on distribution changes, Tertiary mammals and birds, Amphibia and reptiles, freshwater fish and invertebrates, Orthoptera, Dermaptera, longicorn beetles, and butterflies. The standard of scholarship is high, but inevitably for most of these groups more can be said of their present than of their past distribution, and we are constantly aware of the central difficulty of reconstructing faunal histories without the sort of fossil records that exist for the mammals, where the story can be best authenticated. Nevertheless, this volume is really valuable in drawing together into a coherent form a very large amount of information, and it represents much concentrated and intelligent research. As a great deal of the material upon which zoogeography is based inevitably accumulates in a rather haphazard manner, through the results of expeditions, museum bulletins, and so on, it is necessary to take stock at intervals and in this sort of way. It does not appear, however, that any very new ideas were brought to the surface by these symposia. This being so, it is a pity that the only index is one of Latin names. Anyone who wants to find other subjects will have to search the 477 pages of text.

To the general reader, perhaps the following four papers are the most useful. P. B. King traces the growth of modern surface features in Western North America, through the comparatively small deposition and mountain movements up to Jurassic times, and then the major upward Cordilleran movements and their aftermath. D. E. Savage gives a thorough conspectus of the fossil land mammals and their bearing upon Western faunal history. R. R. Miller has a very informative paper on the present distribution and origins of freshwater fishes of the West. A large paper on Pleistocene palæo-ecology by P. S. Martin covers a wider scene, including all North America, the West Indies and the Galapagos Islands, and takes in the arrival of man, and his influence on the fauna.

BUTTERFLIES AND MOTHS

Les Papillons

Par Guy Mathot. ("Que Sais-je ?" No. 797.) Pp. 128. (Paris: Presses Universitaires de France, 1958.) n.p.

"LES PAPILLONS" is not 'just another book on Lepidoptera'. There are very few books which aim at giving a popular account of world Lepidoptera and, in spite of the small size of this book (the page size is similar to the "Wayside and Woodland" series), it covers the subject very well.

There are six chapters: from egg to adult; morphology and anatomy of the imago; infra-specific variation; systematic studies on the principal groups of the world fauna; biogeography of the Lepidoptera; summary of biological studies.

The drawings in the book are not of a very high standard and the reproduction is poor, although this may be due to the poor quality of the paper. The text is very detailed in some parts, giving, for example, the chemical composition of various pigments, chromosome numbers and a table of wing loadings (p. 33). Some of the detail could have been left out in favour of other information. The table of wing loading (p. 33) and that (p. 43) giving the respiratory quotient seem unnecessary.

Small errors occur. The classification used (pp. 56–57) is not entirely satisfactory. The use of "Pyralididoidea" and "Pyralididae" instead of the shorter Pyralidoidea and Pyralidae is irritating. A few spelling mistakes have occurred, *A. kühniella* Zell. is *kuehiella* (p. 81). The genus "*Paraponyx*" (p. 81) is always wrongly spelled and should be *Parapoynx* (Hübner, 1825). Some of the nomenclature is out of date (for example, *Lycaena* is used p. 52) instead of *Polyommatus* (Latreille, 1804).

The lack of authors' names after the species anywhere in the book is regrettable. Even in a book popularizing the subject the student should be correctly instructed, and in this case the author's name should have been given, at least where the species was first used.

In spite of these criticisms, the book contains a wealth of information unobtainable in any book of comparative size. It will interest all collectors who wish to study more than just British Lepidoptera.

Paul E. S. Whalley

The Freshwater Life of the British Isles

A Guide to the Plants and Invertebrates of Ponds, Lakes, Streams and Rivers, with an additional chapter on the Vertebrates. By John Clegg. Second edition. (Wayside and Woodland Series.) Pp. 352+67 plates. (London and New York: Frederick Warne and Co., Ltd., 1959.) 21*s*. net.

THIS popular book, first published in 1952, deals in broad outline with the basic information necessary to an understanding of limnology; it has proved its worth as an elementary text for teachers and students and, in particular, for those attending courses at the field study centres.

This new edition has given Mr. Clegg the opportunity to eliminate some errors which had crept into the first edition and to include some new results that had become available since 1952. Some colour plates by Mr. Ernest C. Mansell are noteworthy additions, those on Mollusca being particularly good. The opportunity might also have been taken to provide more satisfactory colour photographs both of the natterjack toad and of the common toad.

Although a great many animals and plants can be identified with the aid of this book, there are no keys, and it is not primarily concerned with identification down to species. Illustrated extended keys (of too recent date to have been included in the revised bibliography) are now available and can be used in conjunction with this volume, but they do not pretend to provide a comprehensive, well-balanced introduction to limnology such as the beginner will find in Mr. Clegg's book. W. J. REES

The Future of Non-Ferrous Mining in Great Britain and Ireland

(Proceedings of a Symposium arranged by the Institution of Mining and Metallurgy in collaboration with the United Kingdom Metal Mining Association, held on 23rd and 24th September, 1958, at the Federation of British Industries, London.) Pp. xxxvi+614. (London: The Institution of Mining and Metallurgy, 1959.) 60*s*.

THE main scientific results of the symposium organized jointly by the Institute of Mining and Metallurgy and the United Kingdom Metal Mining Association have already been reported briefly in *Nature* (Oct. 25, 1958, p. 1136). Publication of the Proceedings, delayed by the printers' strike, has now been achieved. The single volume contains the text of the fourth Sir Julius Wernher Lecture, delivered as a prelude to the symposium by Mr. Anton Grey. This is followed by thirty-two original papers, most of which had been issued as pre-prints to members, together with verbatim reports of the discussions at the two days of meetings. Sixteen of the papers are devoted to descriptions of the tin, copper, lead, zinc, fluorine and barium deposits of the British Islands, the most comprehensive review of the epigenetic mineral deposits of Britain so far assembled in a single volume. History, geology, production and potential are dealt with in each of these papers. Most of the ore-fields have been described before, some of them in some detail, as for example in the Geological Survey Memoirs of the Northern Pennine Ore-field (1948) and South-Western England (1956), some only in a general fashion. The present publication gives an up-to-date picture of an industry which is now no more than a shadow of its former self. The purpose of the symposium was to consider whether resources of the non-ferrous minerals remain in the British Isles after more than 2,000 years of exploitation; whether these are worth finding, and if so how they can be located. With these points in view, the other papers and discussions were devoted to modern techniques for prospecting—geophysical, geochemical, radiometrical—and to considerations of economics and ownership. If the conclusions can be summed up in a single phrase, they are that deposits as yet undiscovered may be expected to remain: that in all but a few cases the search for these would be so speculative as to need some form of State support; and that when they are found the value of the deposits against the current economic background cannot be said to be assured.

K. C. DUNHAM

Infant Mortality in India, 1901–55

A Matter of Life and Death. By Prof. S. Chandrasekhar. Pp. 175. (London: George Allen and Unwin, Ltd., 1959.) 20*s*. net.

IN writing this book Prof. Chandrasekhar essays the difficult task of being three persons at once. As director of the Indian Institute for Population Studies he makes clear the inadequacy of available statistics. Though the wealth of material in the decennial censuses of India since 1881 is well known, essential basic data such as total number of births, or total number lost under one year, are not available. In the second place the author, as a distinguished social scientist, has little difficulty in showing how unfavourably India compares with so many other countries. Though the infant mortality-rate is shown as having dropped from 215 per 1,000 live births in 1901–05, to 116 in 1951, the latter figure is compared with such examples as 29 in the United States or 27 in England and Wales. In fairness it is noted that infant mortality is still believed to average 300–500 per 1,000 live births over most of Africa. In the third part of the book the author, as social reformer, claims that the nation's children are too precious to be wasted by premature death, that the nation's efforts should be directed to the ideal of reducing the present infant mortality-rate to something like 20 per 1,000 live births. Family planning with a limitation to, "say, three under normal circumstances spaced, say, three years apart," is mentioned incidentally rather than made a focal point.

L. DUDLEY STAMP

Second Conference on Co-ordination of Galactic Research

Edited by A. Blaauw, G. Larsson-Leander, N. G. Roman, A. Sandage, H. F. Weaver and A. D. Thackeray. (International Astronomical Union Symposium No. 7, held at Saltsjöbaden, near Stockholm, June 1957.) Pp. viii+93. (Cambridge: At the University Press, 1959.) 15*s*. net.

THE first conference on the co-ordination of galactic research was held in 1953 and its report appeared as International Astronomical Union Symposium No. 1; it was devoted to a broad survey of the problems of galactic structure. The second conference was devoted to more detailed problems of particular importance at this time: clusters and associations, the Galaxy as a whole, and the Magellanic Clouds. A good account is given of many aspects of current work as well as discussions of the possibilities for future investigations. Emphasis is laid on the importance of adequate standardization of the photometric systems used by different observers.

ROLE OF INFECTIVE NUCLEIC ACID IN THE PRODUCTION OF ENCEPHALOMYOCARDITIS VIRUS

By Dr. F. K. SANDERS

Director, Virus Research Group, Medical Research Council Laboratories, Carshalton, Surrey

COLD phenol treatment[1] of cells infected by several different animal viruses yields infective material, a major constituent of which appears to be ribonucleic acid[2-7]; in this article such preparations will be referred to as 'infective ribonucleic acid'.

In some cases, infective ribonucleic acid seems to come from the virus itself; but in others to form part of another component, which does not sediment appreciably under conditions which bring down virus[4,7]; in the latter cases phenol at 4° C. fails to extract infective ribonucleic acid from the virus particles themselves, although hot phenol (40° C.–60° C.) may be able to do so[7]. The extracellular fluid into which virus has been released at the end of virus growth in mouse ascites tumour cells infected *in vitro* with murine encephalomyocarditis virus contains a component, sensitive to cold phenol, which is not the virus itself[8]. Such a component might be: (*a*) some kind of cellular structure formed as a result of infection, in relation to which virus is synthesized; (*b*) a precursor of the virus particle itself; (*c*) a product of the abnormal synthesis, which somehow fails to be incorporated into a virus particle; (*d*) a breakdown product of virus particles.

Continuously agitated suspensions of Krebs 2 mouse ascites carcinoma cells, infected with encephalomyocarditis virus *in vitro*[8], were used to follow the appearance of infective ribonucleic acid during virus growth, extracts being made from cells at all stages of the virus growth-cycle up to the beginning of virus release. Infected cells, washed with phosphate-buffered saline, and resuspended at a concentration of 5×10^7/ml. in 0·02 *M* phosphate *p*H 7·2, were extracted three times with water-saturated phenol. Phenol was removed from the aqueous layer with ether and the ribonucleic acid precipitated by ethanol. The precipitate was washed three times with 67 per cent ethanol/33 per cent 0·14 *M* sodium chloride and redissolved in 0·2 *M* phosphate buffer *p*H 6·8. All operations were carried out at 4° C. and all aqueous solutions except the 0·2 *M* phosphate buffer contained nickel ions to stabilize the ribonucleic acid; the optimal concentration of nickel was found to be 1 nickel ion per nucleotide, as already found for tobacco mosaic virus ribonucleic acid[9]. A small residual precipitate of nickel phosphate was removed by centrifugation. Ribonucleic acid preparations were mixed with tumour cells and titrated in mice[8], on the day of preparation, although the above method gave a product which retained its original titre for at least 14 days at 4° C., and 24 hr. at 20° C. (Vizoso, A. D., and Burness, A. T. H., personal communication).

In multiply infected cell populations (cells 1×10^7/ml.; virus 3×10^7/ml.) at 37° C. infective ribonucleic acid could be found as early as 15 min. after infection. Thereafter the level of infectivity rose to a maximum at about 5 hr., when 100–1,000 times the amount present initially was found. Infective ribonucleic acid production is thus practically complete by the time new virus is formed; the latter appears intracellularly at about 4 hr., and then increases to maximum titre 7–8 hr. after infection. Cells begin to die and release this virus 6–7 hr. after infection, and release from the whole population is virtually complete at 12 hr. The extracellular fluid then contains more than 2×10^9 plaque-forming units of virus/ml. and a similar number of mouse ID_{50} when titrated *in vivo*.

The increase in infective ribonucleic acid content found during the first hour probably does not involve synthesis of new ribonucleic acid, but represents the formation of an increasing number of virus/cell complexes, in which the ribonucleic acid of the infecting virus becomes capable of extraction by phenol, for cells infected for 2–16 hr. at 4° C. all contain ribonucleic acid with an infectivity corresponding to the level reached in 1 hr. at 37° C., and this level of infectivity is also reached in the presence of inhibitors (see below). Similarly, a fall of about 90 per cent in the infective ribonucleic acid content occurring between 5 and 7 hr. after infection (Fig. 1) need not involve the destruction of infective ribonucleic acid but its progressive inclusion in virus particles whence it cannot be extracted by cold phenol in an infective form.

Effect of Euflavine

Experiments in which virus was grown in the presence of euflavine gave further information about the relationship of the early appearance of infective ribonucleic acid to virus synthesis. In an initial experiment cells (10^7/ml.) were incubated for 1 hr. in the presence of various concentrations of euflavine,

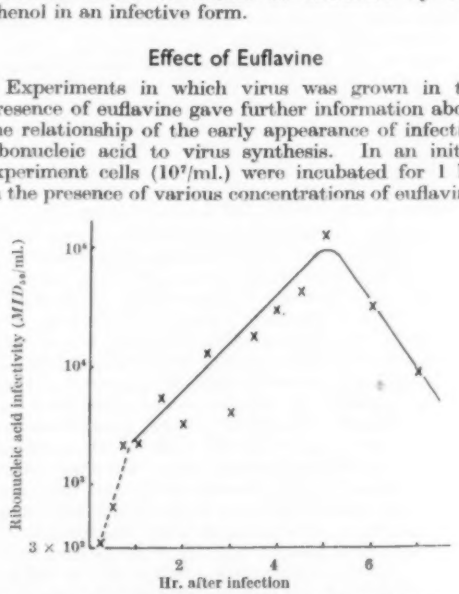

Fig. 1. Infectivity of 'cold phenol ribonucleic acid' at intervals during virus growth

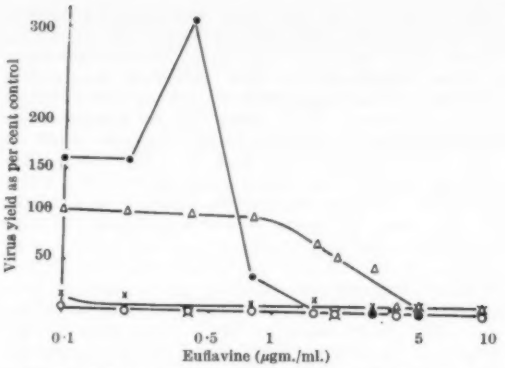

Fig. 2. Effect of various concentrations of euflavine on virus production. ○, plaque-forming units; ●, hæmagglutinin; ×, infective ribonucleic acid; △, cell mortality

all of which were without significant toxic effect upon control uninfected cultures in experiments of similar duration. Virus was then added (multiplicity = 4), and the culture incubated for 16 hr., when the following quantities were measured; (*a*) virus hæmagglutinin concentration, and (*b*) concentration of infective virus (plaque-forming units), in the supernatant fluid; (*c*) the infectivity of ribonucleic acid extractable from the whole culture (cells plus supernatant); (*d*) the number of cells killed by virus multiplication[6,8,10].

All euflavine concentrations tested, even the smallest (0·1 μgm./ml.) virtually suppressed the production of infective virus (Fig. 2); hæmagglutinin production was also inhibited by euflavine concentrations greater than 2 μgm./ml. However, in low concentrations (0·1–0·5 μgm./ml.), hæmagglutinin production was high, probably somewhat increased with respect to the control. Proflavine, a related drug, interferes with a late stage of bacteriophage multiplication—probably that of virus assembly[11,12]—and it is possible that low concentrations of euflavine can similarly disturb the multiplication of encephalomyocarditis virus. Even small amounts of euflavine significantly reduced infective nucleic acid formation, although high doses of the drug failed to reduce the

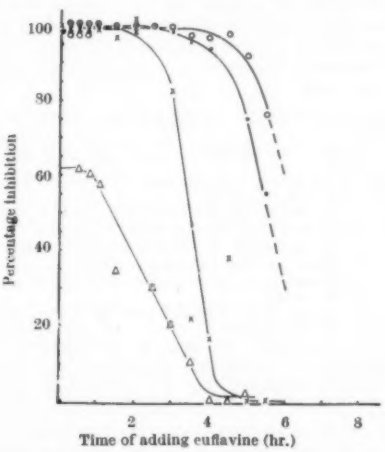

Fig. 3. Effect of adding 5 μgm./ml. euflavine at different times during virus growth. Symbols as in Fig. 2

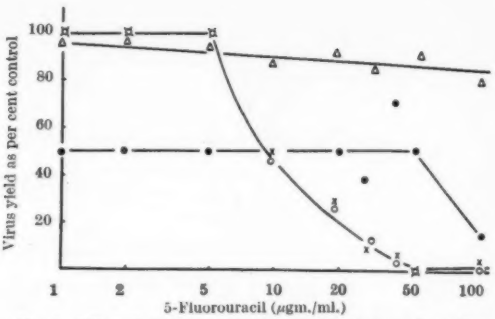

Fig. 4. Effect of various concentrations of 5-fluorouracil on virus production. Symbols as in Fig. 2

infective ribonucleic acid content below that found in untreated cultures during the first hour after infection. With less than 1 μgm. euflavine/ml. as many cells eventually died as in the control, but in higher concentrations more and more cells appeared to resist the lethal effect of the virus, so that at concentrations greater than 4 μgm./ml., virtually all cells were preserved.

Fig. 3 gives the result of an experiment in which 5 μgm./ml. of euflavine was added to ascites tumour cell suspensions at different times after infection. Up to 1½ hr. this dose of euflavine prevented the production of all virus-related substances measured. When given at 4 hr., however, while the production of hæmagglutinin and plaque-forming units was still suppressed, the production of infective ribonucleic acid was normal and the cells died at the proper time. When euflavine was given later than 4 hr., more and more hæmagglutinin was found although even at 5½ hr. after infection there was still significant inhibition of the production of complete virus particles (plaque-forming units). Thus the production of infective ribonucleic acid must be virtually complete by the time hæmagglutinin begins to appear; the latter appears only to be incorporated into plaque-forming virus particles towards the end of virus growth.

Effect of 5-Fluorouracil

This pyrimidine analogue can become incorporated into the ribonucleic acids of animal tissues[13], bacteria[14] and tobacco mosaic virus[15] and it was thus of interest to discover what effect it might have on encephalomyocarditis infective nucleic acid and virus production; all concentrations tested were without significant toxic effect upon the cells under the conditions used. Doses of 5-fluorouracil up to 20 μgm./ml. had little effect on the production of hæmagglutinin and failed to prevent virus-infected cells from dying at the end of the virus growth-cycle (Fig. 4). However, with doses greater than 5 μgm./ml. there was a progressive diminution in the number of plaque-forming units, so that at 50 μgm./ml. or more, less than 1 per cent of the normal number was found. High levels of the drug also affected the production of hæmagglutinin although to a smaller extent. The curve of suppression of infective ribonucleic acid production was very similar to that for plaque-forming units, which suggested that infective ribonucleic acid might actually become incorporated into the virus particle. In further experiments 100 μgm./ml. 5-fluorouracil was added at various times after infection (Fig. 5).

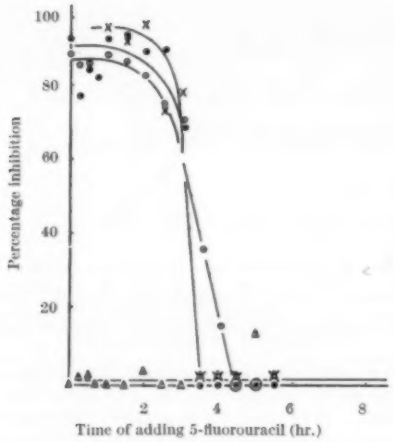

Fig. 5. Effect of adding 100 μgm./ml. 5-fluorouracil at different times during virus growth. Symbols as in Fig. 2

When this amount of 5-fluorouracil was given 1 hr. before virus, there was marked inhibition of hæmagglutinin production and the production of infective ribonucleic acid and plaque-forming units was suppressed; but there was no sparing of the cells. Up to 1 hr. after infection, its effect was the same. With later administration of the drug, however, more infective ribonucleic acid, more hæmagglutinin and more infective virus particles were produced. Addition of the drug at any time after 4 hr. failed to affect the course of virus multiplication. 5-fluorouracil presumably acts during nucleic acid synthesis by replacing part of the uridylic acid residues with their 5-fluoro analogue[14,15]. Anomalous nucleic acid containing this substance is presumably not infectious, and virus particles with such a nucleic acid would not be able to reproduce themselves. The fact that the time-course of suppression by 5-fluorouracil is the same for both these entities suggests that the ribonucleic acid formed between 1 and 4 hr. after infection eventually becomes part of the virus particles. Moreover, since hæmagglutinin production is only significantly affected by 5-fluorouracil when the drug is given during infective ribonucleic acid formation suggests that the newly synthesized ribonucleic acid may act as a centre for the later synthesis of hæmagglutinin.

The multiplication of encephalomyocarditis virus thus probably takes place as follows. Contact of a virus particle with its host cell causes the formation of a virus/cell complex; this can be defined operationally as some alteration in the virus structure resulting from contact with the cell which releases the virus ribonucleic acid in a form extractable by phenol at 4° C. Complex formation presumably involves neither nucleic acid nor protein synthesis since it is not inhibited by either fluorouracil or euflavine, and takes place easily at 4° C. and at 37° C. The next stage seems to be replication of ribonucleic acid carrying the genetic information of the virus, which probably begins at about one hour after infection and reaches a maximum about four hours later; it gives rise to the component described earlier[6], which is the source of the infective ribonucleic acid extracted by cold phenol from infected cells. Only later comes the production of virus hæmagglutinin from $3\frac{1}{2}$ to 6 hr. after infection, and this stage can still be inhibited by euflavine added 4 hr. after infection, though not by 5-fluorouracil added at this time. In the final stage of virus reproduction a core containing the nucleic acid and the hæmagglutinin are put together to make a virus particle. This phase can be disturbed by concentrations of euflavine which are too small to affect hæmagglutinin production. The ribonucleic acid component thus seems essentially to be a virus precursor. Besides its possible function as a centre for the later synthesis of hæmagglutinin, it may eventually become incorporated into the virus particle itself. The process of incorporation is not, however, 100 per cent efficient and a proportion of the component fails to get included in virus particles; it is this extra material which gives rise to the infective ribonucleic acid that can be extracted from the extracellular fluid at the end of virus growth.

It has been stated elsewhere[6] that infection of a cell by virus starts two processes—one resulting in the production of progeny virus, and the other in cell death. The train of events which leads to death of the cell seems to depend on some key change which takes place very early in the growth-cycle. Procedures which favour the production of virus containing anomalous nucleic acid, interfere with the synthesis of hæmagglutinin, or the assembly of virus particles, do not normally prevent cell destruction at the proper time. Blocking virus synthesis by adding 5 μgm./ml. euflavine within $\frac{1}{2}$–1 hr. of infection preserves the cells from eventual destruction, which suggests that the irrevocable event which eventually leads to cell death may be the start of synthesis of the ribonucleic acid component itself. However, evidence from analysis of the interaction between encephalomyocarditis virus strains of narrow host-range, and ascites cells with which they are unable to form plaques, indicates that this may not be so. Cells of the Ehrlich carcinoma or sarcoma 37 are able to take up encephalomyocarditis virus of mouse brain origin[8,16], although new hæmagglutinin or plaque-forming virus is not produced as a result of infection. Large amounts of infective ribonucleic acid—as much as is produced by the fully susceptible Krebs 2 cells—can be extracted from such abortively infected Ehrlich or sarcoma 37 cells, and a component containing this ribonucleic acid is set free into the external medium. Such cells do not die as a result of infection, so death of a cell need not be an inevitable consequence of synthesis of viral ribonucleic acid.

[1] Schuster, H., Schramm, G., and Zillig, W., *Z. Naturforsch.*, **11**, *b*, 339 (1956).
[2] Colter, J. S., Bird, H. H., Mayer, A. W., and Brown, R. A., *Virology*, **4**, 522 (1957).
[3] Alexander, H. E., Koch, G., Mountain, I. M., and Van Damme, O., *J. Exp. Med.*, **108**, 493 (1958).
[4] Wecker, E., and Schafer, W., *Z. Naturforsch.*, **12**, *b*, 415 (1957).
[5] Franklin, R. M., Wecker, E., and Henry, C., *Virology*, **7**, 220 (1959).
[6] Huppert, J., and Sanders, F. K., *Nature*, **182**, 515 (1958).
[7] Schafer, W., *Symp. Soc. Gen. Biol.*, **9**, 61 (1959).
[8] Sanders, F. K., Huppert, J., and Hoskins, J. M., *Symp. Soc. Exp. Biol.*, **12**, 123 (1958).
[9] Cheo, P. C., Friesen, B. S., and Sinsheimer, R. L., *Proc. U.S. Nat. Acad. Sci.*, **45**, 305 (1959).
[10] Sanders, F. K., *Proc. Roy. Soc. Med.*, **50**, 911 (1957).
[11] Foster, R. A. C., *J. Bacteriol.*, **56**, 795 (1948).
[12] Kellenberger, G., and Kellenberger, E., *Virology*, **3**, 275 (1957).
[13] Chaudhuri, N. K., Montag, B. J., and Heidelberger, C., *Cancer Res.*, **18**, 318 (1958).
[14] Horowitz, J., and Chargaff, E., *Nature*, **184**, 1213 (1959).
[15] Gordon, M. P., and Stachelin, M., *Biophys. Biochim. Acta*, **36**, 351 (1959).
[16] Hoskins, J. M., *Symp. Soc. Gen. Microbiol.*, **9**, 122 (1959).

FALL-OUT OVER NORWAY FROM HIGH-YIELD NUCLEAR EXPLOSIONS

By T. HVINDEN, D. HVEDING, A. LILLEGRAVEN and S. H. SMALL

Division of Physics, Norwegian Defence Research Establishment, Kjeller

FROM measurements made in Norway during the past three to four years it seems that fall-out materials which rise well into the stratosphere after high-yield test explosions begin to reach the lower stratosphere over south-east Norway shortly afterwards. The radioactivity in the lower stratosphere seems to increase to a peak about one to two months after the explosions and then to decrease with an effective half-life of about a month. This is shown in Fig. 1, curve *A*, the arrows indicating the delay times. The radioactivity of fall-out materials in air near the ground also begins to increase shortly after the explosions, but more slowly, and seems to reach a peak, which is considerably lower than the peak in the lower stratosphere, after about six to seven months. This is shown in Fig. 1, curve *B*, the delay times being once again indicated by arrows. The rate of increase apparently results in a doubling of the radioactivity in the course of a month or so. The subsequent rate of decrease appears to be the same as in the stratosphere, the radioactivity being halved about once every month. The radioactivity of fall-out materials in precipitation begins to increase at about the same time as the radioactivity of air near ground-level, and follows very much the same pattern. This is shown in Fig. 1, curve *C*.

An interesting observation from Fig. 1 is that the rates of decrease of radioactivity in the atmosphere after large additions of fall-out materials during the years 1957–59 have been remarkably alike, regardless of the overall amounts of fall-out materials in suspension. By correcting for radioactive decay, it can be calculated that the effective residence half-life of fall-out materials in the lower stratosphere over south-east Norway has been of the order of three months.

Since fall-out materials may begin to arrive in air near ground-level shortly after high-yield explosions, it follows that during periods when explosions occur at relatively short intervals there may be considerable mixing of materials near the ground. This can be illustrated by the position at the end of

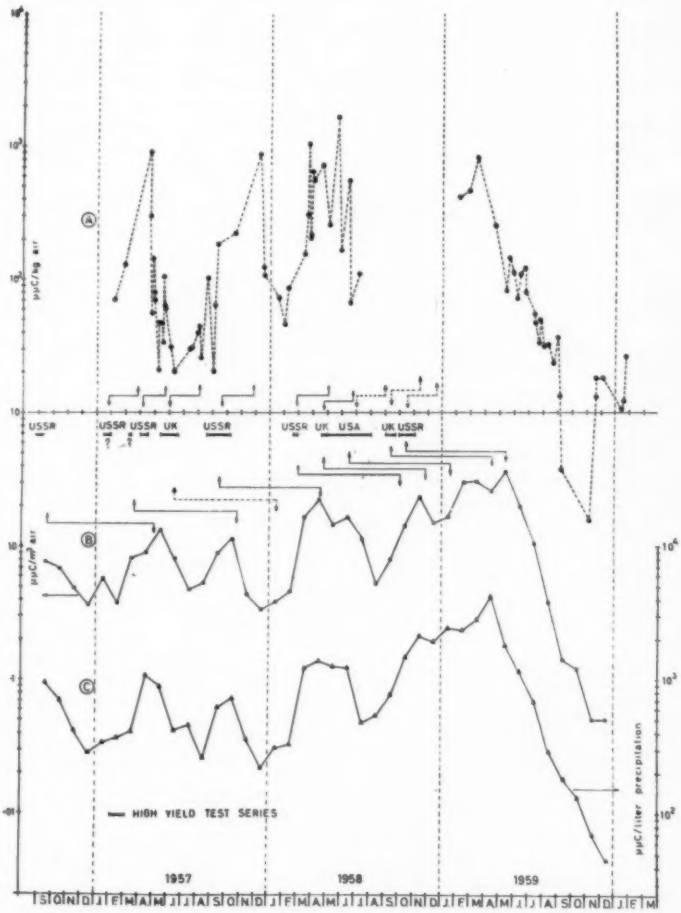

Fig. 1. Results of measurements of fall-out materials during the period September 1956–January 1960
Curve *A*, concentrations of fall-out materials in the lower stratosphere over south-east Norway. Each point represents the result of one measurement. Curve *B*, monthly average concentrations of fall-out materials in air near ground-level. Each point represents an average value of measurements from nine stations. Curve *C*, monthly average concentrations of fall-out materials in precipitation. Each point represents an average value of measurements from twelve stations

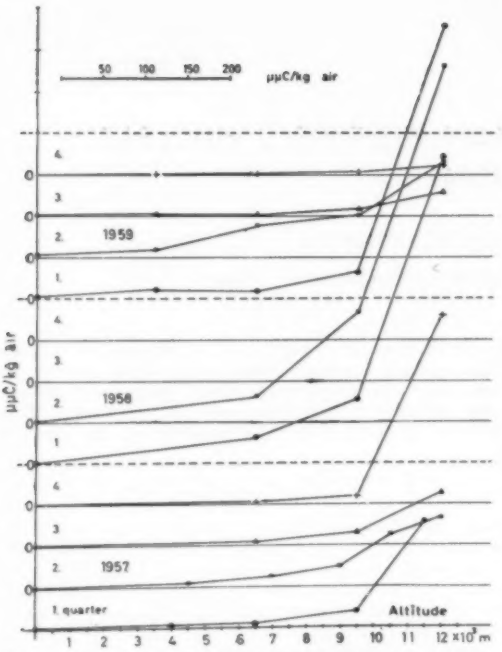

Fig. 2. Concentrations of fall-out materials at different heights above ground-level over south-east Norway during 1957–59. Most points represent the average of 5–10 measurements made during each quarter

1958, when materials from at least five test explosion series could have been present in significant quantities. It is therefore problematical whether isotope ratios or decay-rates can provide reliable information about the origins of fall-out materials at such times. There is no outstanding seasonal variation in Fig. 1 which cannot be explained by the 'seasonal variation' in test explosions.

It has been found that the concentrations of fall-out materials in precipitation depend to some extent upon amounts of precipitation, which vary very much. Consequently it is not to be expected that curves *B* and *C* of Fig. 1 should be completely alike. Nevertheless, there is a very obvious similarity between them, and it can be deduced that on the average 1 cm.³ of precipitation has contained about the same amount of fall-out materials as 1 m.³ of air near ground-level.

The curves shown in Fig. 2, which indicate the way in which concentrations of fall-out materials vary with height above sea-level, might at first sight be interpreted as indicating that downward transport of fall-out materials is hindered by the tropopause layer. However, it seems very possible that the marked increase in concentrations at about tropopause height is due to the rapid removal of materials from lower air layers. Fig. 2 would then indicate that removal processes are effective up to heights of several km. By comparing measurements of airborne materials with measurements of deposited materials, it has been calculated that it would have been necessary to remove the total content of fall-out materials in the troposphere over south-east Norway about once every month, in order to account for the measured deposition. This implies a rather rapid rate of supply to the troposphere, and is interesting in connexion with the short residence half-life of fall-out materials in the lower stratosphere deduced from Fig. 1.

Fig. 3 shows that the main variations in concentrations of fall-out materials in surface air occur approximately simultaneously at measurement sites from Vadsø in the north to Sola in the south, a distance of about 1,700 km. This is quite interesting because the sites are at different heights above sea-level, and are exposed to markedly different weather conditions. It seems clear from Fig. 3 that if fall-out materials are mainly removed from the stratosphere by downward air movements, then these must occur rather consistently over a wide region.

Although much more work must be done to find out what happens to fall-out materials after a nuclear explosion, it now seems that deposition is a fairly continuous short-term process, lasting months instead of years, and affecting very large areas. It is possible that fall-out particles may achieve significant settling-rates, particularly in the lower stratosphere and upper regions of the troposphere. Removal from the stratosphere by downward air movements would then be relatively less important than previously assumed.

In view of the short deposition times, measurements of external radiation-levels and gross activity

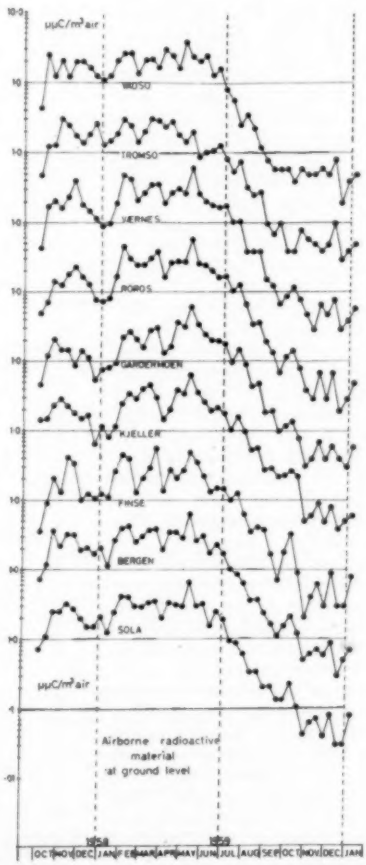

Fig. 3. Mean measurements over ten-day periods of concentrations of fall-out materials in air near ground-level at different stations in Norway during October 1958–January 1960

measurements now have an increased importance, in relation to measurements of selected long-lived isotopes, in assessing possible radiation hazards.

The investigations described in this article form part of a programme of fall-out measurements carried out by the Norwegian Defence Research Establishment. We wish to acknowledge the help given by the Royal Norwegian Air Force and the Norwegian Meteorological Service in collecting samples of fall-out materials, and by members of the Physics Division of the Norwegian Defence Research Establishment in measuring samples, etc.

RADIOACTIVE MATERIALS AND THE SKELETON

THE one-day conference organized by the Bone and Tooth Society on "Radioactive Materials and the Skeleton" included papers on tracer applications of bone-seeking isotopes in biology and in medicine, studies of their biological effects, measurements on rates of deposition of strontium-90 in fall-out, the entry of strontium-90 into food, a survey of the radiation doses received by man from environmental sources and some discussion on the biological implications of environmental radiation in so far as bone was concerned. It was, in fact, a very ambitious programme for a short meeting, but it served the very useful purpose of allowing this controversial subject to be seen as a whole.

The first paper, "Anatomical Sites of Deposition of Radioactive Materials in Bone", was given by Prof. P. Lacroix, of the University of Louvain, who has himself been responsible for many fundamental advances in the field of the dynamics of bone growth. He showed calcium-45 autoradiographs of bones of young and adult dogs and discussed the relevance of the variations in the uptake of calcium throughout the bone to our understanding of the various processes of bone growth and remodelling which take place. Of particular interest was the change in the pattern of uptake with age of the young animal. Soon after birth the major mode of transverse growth of the long bones is by the peripheral deposition of thick layers of bone, the structure of which is still undetermined. This, however, gives way to transverse growth mainly by a process of deposition of rows of osteones under the periosteum, with a different pattern of calcium uptake, which is clearly demonstrated by autoradiography.

In clinical work with bone-seeking isotopes the material for autoradiographic investigation is generally not available and the distribution of the administered isotope must often be determined by external counting methods, which give relatively poor resolution. Much less information is derived than from experimental biological studies but still, as the second paper of the conference, "Bone-seeking Isotopes as Indicators of Bone Turnover", by Drs. G. Bauer and Bo Wendeberg, of Malmö, showed, information can be obtained on the turnover of bone mineral in pathological conditions of bone which cannot be obtained by other methods.

A study of the metabolism of strontium, with strontium-85, in human subjects using two volunteers, was next described by Dr. G. E. Harrison, of the Medical Research Council Radiobiological Research Unit, Harwell, in his paper, "The Skeletal Retention of Radioactive Strontium in Man following an Intravenous Dose". Although a dose of only 0·5 μc. strontium-85 was given to each subject, it was possible to follow the retention pattern for one year, first using excretion data and then measurements on retained activity by whole-body counting. The results for the two subjects were remarkably similar, demonstrating the early initial excretion and then a period of relatively rapid loss with a half-life of about 50 days followed by a slow loss from the relatively unexchangeable part of bone. The calculated mean radiation dose to the skeleton over a period of 300 days was 0·23 rad.

The three papers of the second session dealt with the biological effects of bone-seeking radioactive isotopes. There is a considerable literature on this subject relating mainly to rates of tumour incidence with different isotopes. Large differences in tumour incidence have been found with different isotopes and different patterns of administration ; but the significance of such differences are obscure without information on the distribution of radiation dose in bone and its variation with time, and such information is generally not available.

The first two papers both discussed the histological changes in the bones of growing animals in relation to measured radiation dosage distribution. The first paper, "The Relation of Radiation Damage to Radiation Dose from Strontium-90", by Drs. Sheila Macpherson and Maureen Owen, of the Medical Research Council Group for Research on Bone-Seeking Isotopes, Oxford, dealt with the effect of a long-lived isotope in rabbits, whereas the second, "Bone Tumour Production : Dose Response Relationships using Phosphorus-32", by Drs. J. P. M. Bensted and N. M. Blackett, of the Physics Department, Institute of Cancer Research, London, discussed the effects of a relatively short-lived isotope in rats. In spite of these differences there was much similarity in the gross biological effects observed and both papers demonstrated the considerable reparative capacities of bone to large radiation doses, of the order of thousands or even tens of thousands of rads. With regard to possible histological antecedents of bone tumour production the two sets of experiments had less in common. Whereas with repeated doses of phosphorus-32, which is a particularly efficient method of producing bone tumours in the rat, an increase in connective tissue in the metaphysis appeared to be closely related to bone tumour production, the experiments with strontium-90 did not demonstrate this. In both sets of experiments the tumours observed arose after very heavy radiation doses, of the order of thousands of rads.

These two papers pointed to the work that remains to be done in this field, rather than to what has been achieved. Certainly the mechanisms of bone tumour production by radiation and the likelihood of bone tumours appearing at low dosage-levels are not yet subjects for easy speculation.

Abnormality in tooth development of young animals given large doses of bone-seeking isotopes is a common finding in experimental work, but relatively little work has been done on the histological

changes involved. Prof. M. A. Rushton, of Guy's Hospital Medical School, in his paper, "Damage to Dental Tissues in High-Dose Rabbit Experiments", presented a detailed histological study of the response of the developing teeth of weanling rabbits given large doses of strontium-90 (600–1,000 μc./kgm.). The damage observed was very severe; but the radiation doses were extremely high, of the order of tens of thousands of rads. It is greatly to be hoped that work of this calibre can be extended to much lower levels of radiation dose.

The third session was devoted to problems of environmental radioactivity where the radiation dose-rates involved are of the order of a fraction of a rad a year. The first two papers presented dealt with the measurement, and some aspects of the metabolism, of strontium-90. In order to measure the amounts of strontium-90 deposited in Great Britain from fall-out and the resulting labelling of our foodstuffs a considerable organization has had to be built up, whereby samples can be collected from any parts of the country. In their paper, "Strontium-90 in the Environment and in Human Bone", Drs. F. J. Bryant and D. H. Pierson, of Atomic Energy Research Establishment, Harwell, described measurements of strontium-90 content in rain, in drinking water, in human bone and teeth. Apart from the number of test explosions made there are two main factors determining the rate of deposition of strontium-90. The first is the magnitude of the explosions. Debris from explosions of kiloton bombs does not penetrate the tropopause and has a mean residence time in the troposphere of about 30 days. The debris from megaton bombs is, however, injected into the stratosphere and the mean residence time may be as long as 10 years. The second determining factor is the total rainfall over the given period. The strontium-90 content in tap-waters derived from lakes in the United Kingdom in early 1959 was 0·9 μμc./l., with lower values for river waters and wells. The highest strontium-90 content of human bones was found in the age-group 0–5 years and had the average value in the femur of 0·83 μμc./gm. in the second half of 1958.

Dr. R. Scott Russell, of the Agricultural Research Council Radiobiology Laboratory, Wantage, in his paper, "Radioactive Materials in Foodstuffs", discussed the uptake of strontium-90 in plants and vegetation. He stressed that the major part of the strontium-90 contamination of our foodstuffs came from direct deposition of strontium-90 on to the plant rather than via the soil. It could also be shown that there was only a short interval, not more than about two months, between the deposition of strontium-90 and its appearance in milk, the major source of calcium in our diet in Great Britain. Consequently the strontium-90 content of our food was much more dependent on the rate of deposition of fall-out than on the total accumulated amount.

It must have been very clear from these two papers that the information so far obtained on the deposition of strontium-90 in fall-out and its progress through the food chain is the result of a concentration of highly skilled and intelligent effort and has, in the course of the work, opened up new lines of advance in many fields, from meteorology to agriculture. However, the object of the exercise was to learn something of the biological consequences of fall-out, and the measurement of levels of activity can represent only the preliminary step. The translation of these measured quantities into biological effect is the real challenge.

The values of radiation dose-rate in bone resulting from present levels of fall-out was included in the paper given by Prof. F. W. Spiers, of the University of Leeds, on "Radiation Doses to Bone from all Sources". He first dealt with the problem of where in bone one should measure the radiation dose. Clearly the choice must depend on the degree to which the various tissues comprising bone are significant in the development of late effects. This, however, is not known and the significant dosage values must therefore be uncertain, particularly for the alpha-emitting isotopes, on account of their short range. For strontium-90, with its relatively long beta range, there is still a problem, though not so severe as with the alpha emitters.

Prof. Spiers presented tables of the radiation doses received by bone and by soft tissue from cosmic rays and local gamma-rays, from the natural radioactivity of tissues, and from the strontium-90 and cæsium-137 originating in fall-out. Within Great Britain the total dose-rate received from all sources is of the order of 100 millirem per annum, and of this amount cosmic radiation and local environmental radioactivity contribute about 90 per cent. At present levels the strontium-90 contribution to bone does not normally exceed about 4 millirem a year. The contribution to gonadal tissue is extremely small. The major variation in environmental radiation dose received by an individual depends on the gamma-ray contribution from the local environment, but variation in the natural alpha activity of tissues also plays an appreciable part. As a result of these variations in natural radioactivity the dose-rate in bone may vary between individuals by a factor of as much as 2.

Radiation dosage data such as those presented by Prof. Spiers allow one to come to certain conclusions concerning the comparative somatic effects of the various components of environmental radiation. It is difficult, for example, to avoid the conclusion that if present levels of strontium-90 in bone constitute a hazard, a change in locality may incur a much greater hazard. Beyond such comparative statements it is difficult to go, since the biological data relevant to the late radiation changes in bone refer to radiation dose-levels several orders of magnitude higher than that of environmental radiation. Any theoretical treatment of the problem of the somatic effects of small doses involves the widest assumptions—on the part played by specific cell changes, on the significance of gross tissue damage, on the types of tissue at risk and on the part played by the normal homœostatic mechanisms of the body. Nor is it at all certain that a direct attack on the problem, by giving small doses of radiation to large numbers of experimental animals, is justified. In my paper, "The Biological Implications of Radiation Dosage to Bone from Environmental Radioactive Material", I suggested that too much preoccupation with the quantitative aspects of the late effects of low-level irradiation could, in the present state of knowledge, be a great waste of effort and even a hindrance to the better understanding of the subject. It would seem more reasonable to accept the fact of our present ignorance, to rely on comparative radiation dosage data for necessary action and to concentrate experimental effort on a study of the mechanisms, rather than the statistical aspects, of late radiation effects.

L. F. Lamerton

OBITUARIES

Sir Ernest Barker, F.B.A.

THE death on February 17 of Sir Ernest Barker, at the age of eighty-five, means not only the loss of a great scholar and teacher but also the loss of a symbol of what may well seem, in 1960, to have been a happier and more confident world. Ernest Barker always denied being a 'scholar' in the old-fashioned Oxford and Cambridge classical sense. But all that that meant was that he was not one of the elaborately skilful composers in the ancient tongues whom Macaulay (who himself was a master of these dexterities) made fun of. Barker was a very distinguished product of the Oxford 'Greats' school. I suspect that he never lost his conviction, even after many happy years in Cambridge, that for the statesman and for the teacher of statesmen the school of *Literae Humaniores* was the ideal education.

It would be both absurd and unjust to suggest that Barker was an old-fashioned classic incapable of understanding or appreciating the contributions of more modern studies to the science (or as he would have preferred, I think, to put it) the art of politics. He preserved into his old age a great deal of curiosity about modern developments and modern contributions. But it would be also absurd to pretend that he was ever convinced that the modern American statistical analysts of political behaviour and, still more, the modern psychological explainers of political behaviour, have really improved on the study and explanation of the human situation provided by Plato, Aristotle, and St. Thomas Aquinas.

Barker wrote a great many books, all of them meritorious and some very remarkable. His essay on "The Crusades" for the "Encyclopædia Britannica", published as a little book, seems as remarkable to me now as it seemed when, as an undergraduate in Oxford, I had my attention directed to it by the late B. H. Sumner. In his old age he wrote what I am told was an extremely competent book on Byzantium. But I think that his greatest achievement was his "Political Thought of Plato and Aristotle"—a book full of learning and wisdom. I once reproached him for not producing a new edition and almost coaxed him into an admission that he had wasted a good deal of time on less-important things. He did, in fact, produce a new volume on Plato, but never finished the complete revision of this masterpiece. He spent (or, as I think, wasted) an immense amount of learning on a translation of Gierke, which involved the resuscitation of a great many rightfully forgotten Germans. 'C'était magnifique', but it was not what we wanted from Ernest Barker. I think he came to feel this, for he does not cite this laborious and learned and in some ways useful work in "Who's Who".

Barker was a Liberal (both upper case and lower case letters), and that comes out in the admirable little volume he wrote in the Home University Library on English political thought "From Herbert Spencer to To-day" and in his "Principles of Social and Political Theory". He would never have understood that it was possible to separate politics from morals, to reduce politics to statistical description. As much as Dr. Gunnar Myrdal, he would have held that the idea of ethically neutral means was nonsense. Politics was about ethics. If this were not true, it was not worth serious study.

Barker was not only a man of prodigious learning, he was also a man of prodigious energy. At an age when most people think of retirement as a reality, nearly ten years after he had ceased to be professor of political science in Cambridge, he went to Germany, out of a strong sense of mission, to rehabilitate the lost German tradition of academic freedom. It was a task that might have daunted a younger man, and he suffered a very serious illness from which, some think, he never quite recovered, although he continued to work with an energy that would have shamed many men much younger than he was.

In addition to immense intellectual and physical energy, Barker was extremely handsome and had a most magnificent voice, which was made even more effective as it preserved its northern burr. There is a tradition in Peterhouse that he imagined he had lost this burr. Fortunately there was no basis for this illusion. He not only had an academic career of great distinction, but also one of great variety. From Manchester Grammar School he went to Balliol and was successively Fellow of Merton, St. John's, and New College, where he had a most admiring pupil in the late Harold Laski. He was, for a short and not very happy time, principal of King's College, London, and then he was, for a very long and happy time, the first professor of political science in Cambridge and Fellow of Peterhouse. In contemplating Ernest Barker it was easy to think of him as a relic of a dead past. But it was much wiser to think of him as a representative of a great past, of the disciples of T. H. Green (and of Henry Sedgwick), of the moralistic and learned political philosophers of a great era of modern English history. He survived into what he must have thought—and did think—was an evil and distracted world. But he continued to be loyal to both his theory and practice. If sometimes he seemed unreceptive to new ideas, he was totally above the temptations of fashion or current academic prestige. "We shall not look upon his like again."

D. W. BROGAN

Dr. Hsien Wu

DR. HSIEN WU, an authority on blood analysis, died on August 8, 1959. He was born in Foochow, China, on November 24, 1893. He attended a local high school in Foochow and then enrolled in the naval architecture course at the Massachusetts Institute of Technology. After two years, he changed his course to chemistry and was awarded the B.S. degree by the Institute in 1916. After remaining as an assistant in the Department of Chemistry for a year at the Institute, he entered Harvard Medical School in 1917 for graduate study with Dr. Otto Folin in the Department of Biological Chemistry, and received the Ph.D. degree in June, 1919. He continued his researches as a Fellow with Dr. Folin for a further year.

In 1920, Dr. Wu returned to China as assistant in biochemistry at Peiping Union Medical College. After one year, he was raised to an associate and

later placed in charge of the Department. In 1924, he was appointed an associate professor of biochemistry and in 1928 he was raised to professor and chairman of the Department of Biochemistry. He continued as chairman of the Department until January, 1942, when Japan took over the College. In March, 1944, he was called to Chungking to organize the Division of Nutrition and join the National Institute of Health. In July, 1944, the Chinese Government sent him to the United States as its nutrition expert on a special mission to study rehabilitation reconstruction programmes for post-war China. He served as director of the Nutrition Institute, Ministry of Health, until the end of 1947. He was also associated with the Institute as the director of its Peiping Branch during 1946–47.

In 1947, Dr. Wu and five other Chinese scientists were invited by Unesco to attend the International Physiological Congress which was held at Oxford. On his return from the Congress, by way of the United States, in September, 1947, he was extended the privileges of a visiting scholar in the Department of Biochemistry, College of Physicians and Surgeons, Columbia University. In June, 1948, he was appointed a research associate at Columbia. Sensing the political situation in China, he finally made preparations to move his family to the United States.

Dr. Wu joined the Department of Biochemistry, Medical College of Alabama, Birmingham, on September 1, 1949, as visiting professor. Following a heart attack in October, 1952, he retired on September 30 and moved to Boston, Massachusetts, where he devoted his time to higher mathematics, Spanish and writing.

Dr. Wu was a member of an Administrative Committee of three functioning as director of Peking Union Medical College, 1935–37. He was a member of the Chemistry Division of the National Committee on the Standardization of Scientific Terminology during 1921–27; he served as editor of the *Chinese Journal of Physiology*, 1926, and continued on the editorial board until 1942; and was president of the Chinese Physiological Society, 1931–34.

Dr. Wu was a tireless investigator. His bibliography has 160 entries, which includes two books: "Principles of Nutrition" (1929) and "Principles of Physical Biochemistry" (1934). His fields of research included blood chemistry, gas and electrolyte equilibria, nutrition, immunochemistry, proteins and the metabolism of amino-acids using amino-acids labelled with nitrogen-15 as tracer.

In addition to his contribution of the famous system of blood analysis he also was a pioneer in his researches and theory in other major areas. He made quantitative analyses of the precipitate from the antigen–antibody reaction following the use of labelled antigen. In 1928, he used hæmoglobin which was labelled with a coloured group. The next year, he reported the use of iodo-albumin as the antigen and he analysed the antigen precipitin precipitate for iodine and nitrogen content. These were the first analyses of this nature and antedated the use of isotopes for such analyses by several years. He also proposed the first reasonable theory of denaturation of proteins in 1931. Other valuable contributions to protein chemistry by Dr. Wu were his studies on the molecular weights and osmotic pressures of hæmoglobins of experimental animals in aqueous solution, 65 per cent glycerine solution and in concentrated urea. He noted that the molecular weights of oxyhæmoglobins and hæmoglobins of dog and sheep are not changed in urea solution whereas those of ox and horse are reduced to one-half.

Emmett B. Carmichael

NEWS and VIEWS

Pioneer 5

The United States space probe *Pioneer* 5 was launched from Cape Canaveral in Florida at 13.00 U.T. on March 11, to become the third artificial planet of the Sun. The launching missile was a three-stage *Thor-Able* rocket, and the final-stage vehicle attained a velocity of about 6·9 miles per sec. relative to the Earth, in a direction nearly opposite to that of the Earth's motion around the Sun. Its residual velocity relative to the Sun, after escaping from the Earth's sphere of influence, was therefore less than the Earth's orbital speed, and its orbit is consequently smaller than that of the Earth: it is expected to be about 75 million miles from the Sun at perihelion, and about 92 million miles at aphelion, with a period of 311 days. At perihelion it will be near the orbit of Venus, which moves in a nearly circular path at a distance of 67 million miles from the Sun.

Pioneer 5, which weighs 90 lb., has a 5-watt radio transmitter for use near the Earth and a 150-watt transmitter for use at greater distances. The power is provided by solar batteries arranged in four 'paddle-wheel' vanes. To conserve energy, the transmitter is to be switched on for only a few minutes each day, in response to commands from Earth. The radiotelescope at Jodrell Bank is the main tracking station, and the 150-watt signals should be received at distances out to about 50 million miles. If so, *Pioneer* 5 will be within communication distance for about the next five months. *Pioneer* 5 carries various scientific instruments, including a high-energy radiation counter, an ionization chamber and Geiger counters to measure medium-energy radiation, a magnetometer to determine both the strength and direction of the magnetic field in space, and equipment for detecting micro-meteorites.

Pharmacology at University College, London: Prof. F. R. Winton

Prof. F. R. Winton, who is retiring from the University of London chair of pharmacology at University College, was trained originally in chemistry and had been working for some time as an industrial chemist with the Mond Nickel Co. when he decided to take up medicine. He graduated M.D. (Cambridge) in 1926, and he obtained the D.Sc. (London) in 1942. After graduation, he worked with A. J. Clark in the Pharmacology Department of University College, London, and in 1931 returned to the Department of Physiology, Cambridge, first as lecturer and then as reader. In 1939 he was appointed to the London chair of pharmacology at University College, in succession to Prof. J. H. Gaddum. His earliest scientific work, carried out in conjunction with L. T. Hogben, dealt with the identification of a frog melanophore-expanding hormone in the pituitary gland. His sub-

sequent scientific work was concerned mainly with two fields, the physiology and pharmacology of the kidney and of smooth muscle. His chief interest lay in the physical factors controlling the activities of the kidney and he succeeded in establishing and supporting experimentally a series of remarkably simple relations between arterial, venous, ureteric and glomerular pressures from which the capillary glomerular pressure of the mammalian kidney could be calculated. He also worked on the mode of action of diuretics, and during the War he was engaged in a study of the renal damage which followed crush injury in the limbs. Much of his renal work is summarized in a Harvey Lecture given in 1951. Winton's studies on smooth muscle reveal another aspect of his intense interest in the physical aspects of biological processes. They represent one of the earliest systematic studies of 'tonus' and contractility in invertebrate and smooth muscle. During the War, Winton was the only head of a department left in the Medical Faculty of University College and acted as dean throughout. The success of the evacuation of the Faculty to Leatherhead was in great measure due to his personality and drive. He has published several books, among which is the widely used "Text-book of Physiology", by Winton and Bayliss. Winton is a man of varied interests, a connoisseur of wine and a 'cellist of well above average amateur status. He has been throughout, together with his wife, the violinist, Bessie Rawlins, the main inspirer and one of the principal performers of the Chamber Music Club at University College. He has taken a major part in establishing the Student Health Service in the College.

Prof. H. O. Schild

H. O. Schild, who will succeed Prof. Winton, was born in Fiume. He studied medicine in Munich and began his career in pharmacology in Straub's laboratory. In 1932 he went to London to work with Sir Henry Dale at the National Institute for Medical Research. Later, he worked at the University of Edinburgh with I. de Burgh Daly and the late A. J. Clark. In 1937 he went to University College, London, to join Gaddum, with whom he had worked when he first came to England. He was appointed reader in 1947. Working with Gaddum, Schild found that adrenaline in high dilution, in contrast to noradrenaline, gave a strong green fluorescence in alkaline solution. This reaction, modified in various ways, is still the most sensitive physical method for the assay of adrenaline. Schild was the first to infer the presence in extracts of the suprarenal medulla of a second pressor substance, which we now know to be noradrenaline. He developed a method of demonstrating release of histamine in anaphylaxis by estimating the histamine diffusing out of small tissue particles suspended in Ringer solution on the addition of antigen. With this simple and ingenious method he showed with Hawkins and others for the first time that histamine is also released from lungs of asthmatic patients by the specific allergens. In collaboration with Mongar, he became interested in the intracellular reactions with lead to release of histamine. Schild observed that the anaphylactic and allergic contractions of smooth muscles cannot be fully accounted for by the release of histamine, since they occurred also in muscles rendered insensitive to it. We also owe to him the knowledge that strychnine and strychnine methiodide have the property of releasing histamine, one of the earliest demonstrations of histamine liberators; also the finding that inhibitors of diamine oxidase potentiate histamine just as anticholinesterases potentiate acetylcholine.

Pharmacologists value most perhaps his contributions to bioassay. He introduced statistically controlled methods for the assay of substances on isolated tissue preparations, whereby it became possible not only to obtain more accurate results but also to estimate the limit of error in each assay. They are used, for example, for the B.P. assay of oxytocin. He introduced a method for quantitative measurement of the activity of oxytocic drugs on the post-partum uterus in women. In order to ensure greater accuracy when assaying substances on smooth muscles, he designed an automatic method which is widely used. His approach to the study of drug antagonism has been particularly fruitful. It is the pAx method by which drug antagonism can be expressed quantitatively and agonists classified by means of antagonists. Drugs which act on the same receptors give the same pAx value with antagonists. Recently, he made the intriguing observation that drugs contract the fully depolarized smooth muscle, thereby excluding membrane depolarization as the only cause of contraction.

Meteorological Department of India : Shri S. Basu

Shri Basu retired from the Indian Meteorological Service on February 23 after thirty-two years of service in that Department. He was born in the United Provinces on October 23, 1903, where he spent his early years and had a brilliant educational career. He completed his university career at Allahabad under the guidance of the late Prof. Meghnad Saha, whose personality profoundly influenced him as a young student. After completing his post-graduation in physics, Shri Basu entered the Meteorological Department of India in 1927, where he served until his retirement last month. During the period he was director-general of observatories, the Meteorological Department made notable advances in many directions. Apart from his activities in meteorological spheres, he actively participated in some of the other scientific organizations in India; he is a Fellow of the National Institute of Sciences in India and has been the secretary of it during the past four years. He is also a Fellow of the National Academy of Sciences, Indian Physical Society and a member of the Aeronautical Society. He is also the president of the Association of Indian Geographers. He was the founder-president of the Indian Meteorological Society and is now one of the vice-presidents.

In 1951 he was elected vice-president of the Commission for Climatology of the World Meteorological Organization. As representative of India, he led the Indian delegation at the meeting of the World Meteorological Organization in 1955 and 1959 at Geneva. He was a member of the Executive Committee of the World Meteorological Organization during 1954–59. As president of the Regional Association II of the World Meteorological Organization for Asia (1954–59), Shri Basu did a great deal of preliminary work the influence of which will serve to guide the affairs of the Association for many years to come. He has also been India's member in the International Civil Aviation Organization and of the Special Committee of the International Union of Geodesy and Geophysics for the International Geophysical Year.

Shri P. R. Krishna Rao

Shri P. R. Krishna Rao succeeds Shri S. Basu as the director general of observatories, Government of India. He has had long experience of work in the department, having entered the service thirty-three years ago. He has had experience of international meteorological conferences since 1946, when he attended the first International Meteorological Organization Conference of Directors held in London after the Second World War. He recently led the Indian delegation to the joint session of the Meteorological Division of the International Civil Aviation Organization and the Commission for Aeronautical Meteorology of the World Meteorological Organization held in Montreal, and later paid short visits to the Canadian and United States Meteorological Services at Toronto and Washington.

Botany at Belfast : Prof. D. J. Carr

Dr. D. J. Carr has been appointed professor of botany in the Queen's University of Belfast in succession to Prof. J. Heslop Harrison, who has been appointed professor of botany in the University of Birmingham (*Nature*, 173, 150; 1954). Prof. Carr was educated at the University of Manchester after war service with the Royal Air Force. In 1949 he became an assistant lecturer at Manchester, and after a year at the Max Planck Institute at Tübingen, working in association with Dr. G. Melchers, he was appointed a senior lecturer in the University of Melbourne. His promotion to a readership at Melbourne followed in 1959. While at Manchester and Tübingen, Carr was engaged in work on developmental physiology, especially in relation to the photoperiodic responses of flowering plants. Among his contributions in the field of photoperiodism was a demonstration, using grafting techniques, that leaves cannot be photoperiodically induced unless they are in organic connexion with a shoot apex, a result interpreted as favouring Gregory's hypothesis of photoperiodic induction, according to which there can be no accumulation of a 'flowering hormone' in leaf tissues. In Australia, Carr's interests have ranged widely, penetrating into the fields of ecology and taxonomy. He has added to the Australian bryophyte flora a remarkable member of the Marchantiales, *Monocarpus sphaerocarpus* D. J. Carr, a plant of saline flats in north-western Victoria. In association with his wife, Dr. S. G. M. Carr, he has recently begun a major study of the developmental morphology of the inflorescence and flowers of the important genus *Eucalyptus*.

Commonwealth Assistance in Training Teachers

In a written answer in the House of Commons on February 24, the Secretary of State for Commonwealth Relations, Mr. C. J. M. Alport, said that good progress was being made in carrying out the proposals for assistance in training teachers put forward by the United Kingdom at the Commonwealth Education Conference. By the beginning of the next academic year it was expected that more than four hundred additional Commonwealth students would be coming to the United Kingdom to take courses in teacher training institutions with bursaries provided by the United Kingdom Government covering their tuition fees and full maintenance. This was more than half the existing total of Commonwealth teachers already receiving training in Britain. Places would be available for these students in a wide variety of training courses at institutions throughout the United Kingdom, and arrangements for their reception and welfare would be made by the British Council.

Mathematics and Science Teachers in Great Britain

In a written reply to a question in the House of Commons on February 25 regarding mathematics and science teachers, Sir David Eccles said that in 1959 there were 5,100 mathematics and 7,700 science graduates teaching senior pupils in maintained schools, as well as 3,250 non-graduate specialists in these subjects in schools with courses leading to the General Certificate of Education at Ordinary level. Inquiry in 1958 revealed 244 vacancies in these schools for mathematics teachers and 261 for science teachers. Assuming an annual net increase of 500 mathematics and science graduates, the shortage of these teachers on 1959 staffing standards would be 500 in 1961, 750 in 1962, 900 in 1963 and 350 in 1964. In 1965, 250 would be available to improve on 1959 standards. The actual shortage would depend on the results of present measures to increase the number of graduates and the output of specialists from training colleges.

Laboratory Accommodation in British Schools

In reply to a question in the House of Commons on February 11, the Minister of Education, Sir David Eccles, said that about £5 million would be spent in the 1960–61 and 1961–62 major building programmes to improve laboratory accommodation in existing schools; in addition, new schools had the full range of science facilities. He said he regarded this provision as one of the most important elements in the building programme, and in the maintained schools we were building, and would continue to build, every year at a higher rate than ever was spent on the public schools.

The Library Service in Great Britain

Replying in the House of Commons on February 11 to a question regarding the introduction of legislation to implement the recommendations of the Roberts Committee on the Public Library Service, the Minister of Education said that the Government accepted the need for legislation in this field, and he hoped shortly to begin discussions with associations of local authorities on his proposals. He thought it wise to obtain the views of local authorities before the subject was discussed in the House, and agreed that it was necessary to take into account the view that no good local authority that is running a virile library service should be deprived by its size of the right of continuing to do so. Sir David Eccles undertook to bring into his discussions the Smaller Library Group of the Library Association. He indicated that his proposals were more in the sense of retaining the power, authority and responsibility of smaller local authorities, and not transferring all these responsibilities to county councils, and that the library service essentially should be decided by local authorities and not subject to national standards other than the proposals of the report. He also agreed that account must be taken of the possibility of changes in the structure of local government resulting from the recommendations of the Commissions now operating before making any alteration in the library service and that the problem was highly controversial.

Fortieth Anniversary of the Liebig Museum

THE Liebig Museum, Geissen, celebrates this year its fortieth anniversary. Although the Museum was not inaugurated until 1920, the idea had long been in the minds of German scientists and, but for the intervention of the First World War, would have most probably materialized many years before. It was decided at the beginning of the present century to set up as a memorial the Laboratory which Justus Liebig had made so famous. In 1909 a committee was formed for this purpose, including such scientists as Prof. Volhard, who at the time was the last living assistant of Liebig, Prof. Sommer, Prof. Behagel, Dr. Merck and many others. It was from this committee that the Liebig Association emerged in 1911. Much of Liebig's original equipment was transferred from the new Institute to the original Laboratory which he had persuaded the Darmstadt government to provide at Geissen in the early nineteenth century. Repair work was carried out on the Laboratory and much of the lost equipment was copied on the strength of drawings which had been found. The First World War delayed the opening until March 26, 1920; but such was its fate that only twenty years later it suffered further damage by yet another World War. However, after thorough reconstruction, for which funds could only be raised by collections, the Museum was reopened during the summer of 1952; the Institute which had proved to be the mother of chemical institutes all over the world was once more established.

U.S. Federal Funds for Science

THE eighth issue by the National Science Foundation of Federal Funds for Science, dealing with the Federal Research and Development budget for 1958, 1959 and 1960, estimates Federal expenditure on scientific research and development for 1960 at 8,100 million dollars, an increase of 3 per cent over 1959, and compared with a 34 per cent increase in 1959 (pp. iii+74. NSF-59-40. Washington, D.C.: Government Printing Office, 1959. 65 cents). In 1959 the Department of Defence, the Atomic Energy Commission and the National Aeronautics and Space Administration accounted for 6,700 million dollars out of the 7,200 million for research and development. Of the remainder, 244 million dollars was expended by the Department of Health, Education and Welfare, 119 million dollars by the Department of Agriculture, 62 million dollars by the Department of the Interior and 57 million dollars by the National Science Foundation. Profit organizations were responsible for 65 per cent of Federal expenditure on research and development, Federal organizations for 24 per cent, and educational institutions for 9 per cent. Of the estimated expenditure of 1,444 million dollars on research in 1959, 964 million dollars was for the physical sciences, 432 million dollars for the biological, medical and agricultural sciences, and 48 million dollars for the social sciences. Fundamental research accounted for an estimated 488 million dollars in 1959 compared with an actual 331 million dollars in 1958 and an estimated 494 million dollars in 1960; of this, 335 million dollars were for the physical sciences, 142 million dollars for the biological sciences, and 11 million dollars for the social sciences; 49 per cent of the total was expended through educational institutions, 5 per cent through profit organizations and 37 per cent through Federal organizations. Expenditure on research and development plant increased from an actual 342 million dollars in 1958 to an estimated 662 million dollars in 1959 and 447 million dollars in 1960. Of the latter figure, 136 million dollars in 1959 and 92 million dollars in 1962 were on non-Federal sites, and 531 and 355 million dollars, respectively, on Federal sites. Since 1951, basic research, as a proportion of total research, has increased from about one-fourth to almost one-third. In the same period, expenditure on the biological sciences increased by 118 per cent, on the physical sciences by 56 per cent, and on the social sciences by 60 per cent.

Funds for Research in the United States

DATA now available from the U.S. National Science Foundation's continuing survey of funds for research and development in the United States indicate an increase in those funds from just over 5,000 million dollars in 1953–54 to 12,000 million dollars in 1959–60, due primarily to an increase of about 160 per cent in the funds used for research and development by private firms and certain types of related organization which constitute the "industry sector" of the Foundation's programme: for industry the rise is expected to be 3,600 million dollars to 9,400 million dollars, more than half of which now comes from the Federal Government. For colleges and universities the corresponding increase is 126 per cent (450 million dollars to 1,000 million dollars), and for other non-profit institutions, 140 per cent (100 million dollars to 250 million dollars). Taking the completed year 1957–58 in comparison with 1953–54, funds for research and development increased from 5,150 million dollars to 10,050 million dollars, and of these Federal Government agencies provided 6,130 million dollars, compared with 2,740 million dollars; for industry the corresponding figures are 3,660 million dollars and 2,240 million dollars; for colleges and universities, 180 million dollars and 130 million dollars; and for other non-profit institutions, 80 million dollars and 40 million dollars. In terms of performance it is estimated that industry was responsible for 76 per cent of research and development in 1956–57 compared with 70 per cent in 1953–54; Federal Government agencies for 15 per cent, compared with 19 per cent; colleges and universities for 7 per cent, compared with 9 per cent; and other non-profit institutions were still responsible for 2 per cent.

The Metallurgist

BY the issue of the first number of *The Metallurgist*, the Institution of Metallurgists has at last a publication worthy of its growing size and importance (*The Metallurgist*, 1, No. 1; January 1960. Incorporating the *Bulletin of the Institution of Metallurgists*. Pp. 64. Annual subscription, 63*s*. post paid). It is no function of the Institution to duplicate the journals of the other metallurgical institutes, and the contents of this issue are, therefore, concerned either with matters of domestic importance or with articles of general appeal. Among the former may be included the Institution's revised examination requirements, a most carefully carried out piece of work, and reports on the results of the examinations in 1959 and on national certificate results for which it is responsible. The articles comprise Prof. A. J. Murphy's presidential address, "Some Recollections" by Dr. Harold Moore, the first president, and a most interesting article by Prof. R. W. K. Honeycombe entitled "New Horizons in

Metallography", which discusses the applications of the electron microscope and of electron-probe micro-analysis. Dr. Howard K. Warner contributes an account of metallurgy in Australia which will be of great value to any member who contemplates taking up an appointment, teaching or industrial, in that continent. The presentation of the journal is most attractive and the illustrations excellently reproduced, the whole being a credit both to the Institution itself and all those responsible for its appearance.

Science in the School Library

A new edition of "Science Books for a School Library" has been published by John Murray for the Science Masters' Association (1959. 3*s*. 6*d*. net). It contains in its 54 pages three large sections on biology, chemistry and physics, of about 150, 100 and 100 titles, respectively, and smaller lists, on the history of science, astronomy, geology and meteorology. The final section is a list of books suitable for a secondary modern school science library. About half the entries have been reviewed in *The School Science Review*, and the number of the issue is given. Annotations are attempted in a rather half-hearted way, and the need for brevity is no excuse for "a readable book", "a well written history", "a most interesting book", or "packed with information". One wonders why J. B. Conant's "On Understanding Science" is in the chemistry section and hopes that all schools are able to afford Singer's "A History of Technology" (5 vols., £42). The list will be of great use to new schools setting up a library for the first time, or as a check list for an established library. Both users and the editors of the next edition would find of great interest two similar works—"The A.A.A.S. Science Book List" (American Association for the Advancement of Science, 1959) and the National Book League's "Science for All".

Tunnel Diodes

In a recent press release, Standard Telephones and Cables, Ltd., announced the development of a new semiconductor device known as a tunnel diode, believed to be the first of its kind produced in Europe. The main feature of tunnel diodes is that they exhibit a marked negative resistance characteristic, which is likely to have important applications in radio circuit technique. The fact that a germanium diode made of very highly doped material can exhibit a negative resistance for a small applied voltage (100–200 mV.) was first reported by L. Esaki (*Phys. Rev.*, **109**, 603; 1958). The results of a theoretical and experimental examination of tunnel diodes as devices for use at very high frequencies were described by H. S. Sommers, of the R.C.A. Research Laboratories, Princeton, U.S.A. (*Proc. Inst. Radio Eng.*, **47**, 1201; 1959). Diodes with a negative conductance of 1 mho have been made and these oscillate at frequencies above 1 kMc./sec. when incorporated in a special microstrip mounting of low series impedance. Apart from its use as a self-excited oscillator, the tunnel diode, when mounted in a coaxial or microstrip line, can be used as a switch with an operating period of a few milli-micro-seconds. It is believed that a different approach to electronic circuit problems is necessary in order to exploit fully the application of these tunnel diodes; and it is with the object of assisting circuit design engineers to gain experience of these that samples suitable for use at frequencies up to about 5 Mc./s. are being made available in Great Britain by Standard Telephones and Cables, Ltd. Development work is proceeding rapidly, however, and it is expected that devices with a more advanced performance will shortly become available.

High-atmosphere Research in the Meteorological Office

The importance of the continuous study of conditions in the highest parts of the undissociated atmosphere (between about 30 and 100 km.) is now recognized among meteorologists. To further such studies, the Meteorological Office has set up a new assistant-directorate, with responsibilities for both theoretical and experimental research. It is intended to investigate the composition and physical state of the atmosphere up to about 100 km. with special balloons and, it is hoped, small rockets. In addition, the new unit will take over the present design studies for meteorological observations from the second *Scout* satellite. Dr. R. Frith, formerly assistant-director in charge of research in atmospheric physics, has been placed in charge of the unit.

James Clayton Prize, 1959

The James Clayton Prize for 1959 has been awarded to C. Bernard Dicksee, for his pioneering and subsequent development and research work on the high-speed compression-ignition engine, and Prof. G. Fernie Mucklow, for continuous research and experimental work into the propagation of pulses (wave action) in gases, and problems connected with the internal combustion engine. This Prize is awarded annually to a member or members of the Institution of Mechanical Engineers, who contribute most in the year to modern engineering science by way of research, invention, experimental work or a treatise or paper on a modern engineering subject, or originality in engineering design, or by service to engineering.

The Corday-Morgan Medal and Prize

Prof. C. Kemball, professor of physical and inorganic chemistry, the Queen's University of Belfast, has been awarded the Corday-Morgan Medal and Prize for 1958 of the Chemical Society, in consideration of his contributions to the study of heterogeneous catalytic reactions. The award, consisting of a Silver Medal and a prize of 200 guineas, is made annually to the chemist of either sex and of British nationality who has published during the year in question the most meritorious contribution to experimental chemistry, and who has not, at the date of publication, attained the age of thirty-six years. Applications in respect of the award for 1959 must be received by the General Secretary of the Chemical Society, Burlington House, Piccadilly, London, W.1, not later than December 31, 1960, and for the award for 1960 before the end of 1961.

Nature Conservation: Postgraduate Diploma Course

The social, economic and political problems arising from the results of a shortsighted policy for land use have been painfully obvious for a long time. Practical object lessons on a vast scale can still be seen in many parts of the world. Conservation involves the long-term study of land use, and one of the main problems is the prediction of the immediate and ultimate effects of any programme of agriculture or resource exploitation. The solution to these problems will provide the means for avoiding the disasters of the past. One of the main difficulties has been the lack

of trained men, and to meet this need a postgraduate diploma course in conservation will be inaugurated at University College, London, in July. The course will consist of an initial period of field work, extensive and exploratory in nature, followed by two terms of indoor teaching and finishing with a second period of more intensive field work. The course will be run by the Botany Department, in collaboration with Geography and Zoology Departments of University College, with assistance from other departments and a wide range of seminars. The syllabus will include the physical factors of the environment, plant and animal ecology, and a study of different forms of land use and their effect on soils, plants and animals. This fundamental grounding should be applicable to conservation problems in different parts of the world. The Nature Conservancy will award maintenance grants for students to attend the course and will assist in other ways, especially with field work. The diploma will be a useful qualification to those seeking jobs with the Nature Conservancy, or similar organizations in other countries, or with the Colonial Service, or in any other aspect of applied ecology or land use.

Summer School in Radiation Protection

A FORTNIGHT'S summer school of health physics (radiation protection) will be held at the Imperial College of Science and Technology, London, starting on July 4. The course will offer training in radiation protection, at postgraduate-level, for persons who are directly concerned with the subject, and should be of interest to safety officers, medical officers of health, factory inspectors and others concerned with radiation protection. The emphasis will be on practical applications, and training in the measurement of radioactivity will be included. Further information can be obtained from the Registrar, Imperial College of Science and Technology, South Kensington, London, S.W.7.

Royal Society of Tasmania

THE following officers of the Royal Society of Tasmania for the year 1960 have been announced: *President*, His Excellency the Governor of Tasmania, the Right Honourable Lord Rowallan; *Vice-Presidents*, Dr. D. Martin and Mr. R .M. H. Garvie; *Council Members*, Mr. D. H. Borchardt, Dr. C. Craig, Mr. W. F. Ellis, Dr. E. R. Guiler, Mr. L. W. Miller, Mr. K. D. Nicolls, Mr. M. S. R. Sharland and Mr. J. W. C. Wyett; *Hon. Secretary and Librarian*, Dr. W. Bryden; *Hon. Treasurer*, Mr. G. E. A. Hale; *Hon. Auditor*, Mr. A. M. D. Hewer.

The Night Sky in April

FULL moon occurs on April 11d. 20h. 28m. U.T., and new moon on April 25d. 21h. 45m. The following conjunctions with the Moon take place: April 1d. 08h., Aldebaran 0·4° S.; April 16d. 19h., Jupiter 5° S.; April 17d. 21h., Saturn 4° S.; April 22d. 01h., Mars 2° S.; April 24d. 01h., Mercury 1° S.; April 28d. 16h., Aldebaran 0·5° S. Mercury, Venus and Mars are too close to the Sun for easy observation. Jupiter is a morning star, rising at 1h. 40m., 0h. 50m. and 23h. 45m. on April 1, 15 and 30, respectively. It is moving eastwards in Sagittarius until April 20, on which date it is stationary; afterwards it begins to move westwards among the stars. Its stellar magnitude is − 1·9 and its distance from the Earth in the middle of the month is 450 million miles. Saturn is also a morning star, moving eastwards in Sagittarius until April 27, when it is stationary and commences to move westwards among the stars. It rises at 2h. 35m., 1h. 40m. and 0h. 45m. on April 1, 15 and 30, respectively. Its stellar magnitude is + 0·7 and its distance from the Earth on April 15 is 910 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: April 6d. 01h. 00·8m., 29 Cnc. (*D*); April 28d. 15h. 44·0m., Aldebaran (*D*); April 28d. 17h. 03·0m., Aldebaran (*R*); *D* and *R* refer to disappearance and reappearance, respectively. The Lyrid meteors are active during April 20–22; the radiant is at R.A. 18h. 04m., Dec. + 33°, and conditions for observation are fairly favourable.

Announcements

MR. S. S. KIND has been appointed editor of the newly founded *Journal of the Forensic Science Society*. Communications should be addressed to the Editorial Office, c/o Rossett Holt, Pannal Ash Road, Harrogate, Yorks.

THE Bureau of American Ethnology of the Smithsonian Institution has announced recently (Bulletin No. 130) completion of the long awaited index to the volumes entitled "Handbook of South American Indians". The index appears in Vol. 7 (Washington, D.C.: Government Printing Office, 1959).

FOLLOWING an investigation into technical readership by the Department of Scientific and Industrial Research, Heywood and Co., Ltd., are to establish *Electronics Weekly* in September. It will appear as a newspaper and will be directed at manufacturer and user, as well as to management and scientific staff. Further information can be obtained from Heywood and Co., Ltd., Drury House, Russell Street, London, W.C.2.

A SYMPOSIUM on the Matrix will be held by the Bone and Tooth Society at the Nuffield Department of Orthopædic Surgery, University of Oxford, on March 29. Further information can be obtained from B. Cohen, Royal College of Surgeons, London, W.C.2.

THE spring meeting of the Parasitology Group of the Institute of Biology is to be held at the Liverpool School of Tropical Medicine, Pembroke Place, Liverpool 3, during April 6–8. Further information can be obtained from L. P. Joyner, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

AN informal discussion by the Colloid and Biophysics Committee of the Faraday Society on Cytoplasmic Particles and their Role in Protein Synthesis will be held in the University of Reading during March 28–29. Further information can be obtained from Prof. J. A. V. Butler, Chester Beatty Research Institute, Fulham Road, London, S.W.3.

THE Campaign for Nuclear Disarmament announces the formation of a Scientists Group, the purpose of which is to advise the Campaign, in the light of available scientific information, on such subjects as fall-out, civil defence, control of tests, effects of nuclear war, relevant aspects of general disarmament, and civilian uses of nuclear energy. The Group is publishing a regular bulletin, of which the first number (4 pp.) has recently appeared; it includes a number of abstracts of recent scientific papers and publications dealing with fall-out and related topics. Further information can be obtained from the Secretary, Campaign for Nuclear Disarmament, 2 Carthusian Street, London, E.C.1.

UNIVERSITY EDUCATION IN CENTRAL AFRICA

THE events which led up to the granting of a Royal Charter to the University College of Rhodesia and Nyasaland on February 11, 1955, have been reviewed in *Nature* (**172**, 1; 1953. **173**, 107; 1954. **175**, 181; 1955). Now, five years after its establishment, the first results of degree examinations in the College have been announced, and it is opportune to review the five years of life of this flourishing multi-racial establishment. A basic principle in the foundation and planning of the University College was that there should be no discrimination in it on racial grounds, and, as was said in 1955 (*Nature*, **175**, 181; 1955), "In a country where racial tension can arise, the position of a multi-racial University is especially important". The agreement of Her Majesty Queen Elizabeth the Queen Mother to become the first President of the College, and her presence at Salisbury when she was formally installed on July 5, 1957, were particularly happy auguries for the development of this young university institution. As President of the College she will preside at the degree ceremony on May 13, 1960, in Salisbury.

The first academic session opened in March 1957 with a total of 74 students, 8 of whom were African (*Nature*, **179**, 612; 1957). During the session that has just closed there were 168 students, of whom 38 were African. The proportion of African students in the College is steadily rising, and, with the development of sixth-form school education throughout the Federation of Rhodesia and Nyasaland, may be expected to rise still further.

The University College is in special relationship with the University of London and the students therefore now take the degree examinations of that University. At the end of the session which has just closed 19 candidates sat for the B.A. General degree, of whom 17 were successful, one being placed in the first division. Six candidates out of the seven who sat for the B.Sc. General examination, and both of two candidates who took that for B.Sc. Agriculture, were successful in obtaining degrees. In addition, 23 out of 26 candidates successfully completed the course and examination for the postgraduate certificate in education. Of the total number of successful candidates, nine were African.

The examination results to date are a useful index of the soundness of the academic progress of the College. Nevertheless, the needed growth in breadth and depth of educational facilities still depends upon financial encouragement, and it is with great satisfaction that the news has recently been received of an offer of a grant of £250,000 by the Nuffield Foundation towards the establishment of a Medical School at the College. Training in medicine is extremely expensive, and it was natural that the institution of a Medical School should be delayed until a number of other faculties had been established, although this ultimate development was contemplated from the start.

The generous offer from the Nuffield Foundation is made on the understanding that the teaching hospital for the Medical School takes the form of a new multi-racial hospital on the site of the University College. The University of Birmingham and the University College of Rhodesia and Nyasaland have agreed that when a Medical School is established at Salisbury it shall initially be an affiliated institution of the University of Birmingham. This means that, for the period of affiliation, the students of the Medical School of the University College will be prepared for the degrees in medicine of the University of Birmingham just as those in the Faculties of Arts and Science at present prepare for the degrees of the University of London.

As so often seems to hold for university institutions, the financial support of the University College is scarcely adequate for all that, on other grounds, it might hope to do. Her Majesty's Government, which in the past has provided capital money totalling £1,475,000, has recently made a further generous grant of £1,400,000 towards capital costs for the College. The Government of the Federation of Rhodesia and Nyasaland has provided £750,000 over the first five years, for recurrent expenditure, and one may hope that it will give further generous support during the new quinquennium to help to provide for the cost of the much needed expansion which is already planned. At present, besides medicine, the College lacks only engineering and theology among the major faculties to be expected in a university institution.

The working out of a plan for racial partnership in all its details vitally depends upon the co-operation of educated people of all kinds. The significance of education, and particularly the higher education of Africans, in the working out of such a plan must be clear to all.

F. G. Young

ADMINISTRATION OF UNIVERSITIES

THE chuckles in academic circles when Prof. C. Northcote Parkinson presented his famous law to explain certain empire-building propensities of industrial leaders may now be echoed by chortles from industrial leaders who read an article by Parkinson which deals with related matters in universities (*The Universities Review*, **32**, No. 1; October 1959).

Although British universities do not yet boast the organizational luxuriance of American universities—the University of Pittsburg has a chancellor, three vice-chancellors, three assistant chancellors and sixteen directors, eighteen deans and a host of other executives—Parkinson asks whether British universities are not proceeding in the same direction.

A recent study of the expenditure of some fifteen British universities shows that the percentage of their allocation to 'administration' varies between 5 and 10·6 per cent of their annual budget, Bristol spending the least and Exeter bracketed with

Leicester in spending the most. The average is about 7·2 per cent—a not manifestly exorbitant proportion. Expenditure between universities is not an easy comparison to make, for much depends upon the detailed layout of the estimates. Nevertheless, the universities which spend more than 10 per cent might learn something from those which spend only 5 per cent. What these figures do not reveal is the extent to which academic time and effort are diverted to administration on a part-time basis. This is a field in which statistical comparison is more difficult. All that is gathered is a vague impression of middle-aged scholars bewailing the administrative work thrust upon them ; a complaint sometimes intended to excuse their lack of literary or scientific output. Eagerness for office is due to several causes, among which the most important is the least noticed. Presiding over a committee or dictating a routine correspondence is far easier than doing a good piece of research. It may often be better paid and frequently leads to the higher offices and honours, so that the scholar is offered every inducement to desert the library and take to the filing cabinets. Part of the trouble with British university administration centres on this inducement. For those most easily tempted along the easier path are not necessarily the most competent, though many of them prove to be so. In some instances, the burdens of which they complain have become burdensome as a direct result of their own inefficiency.

Those who admire the system of some larger American universities would wish to give more extensive powers to the executive head. There is no assurance, however, that this would be either an improvement or an economy. What is needed is an equivalent of the army 'p.s.c.', a higher qualification to be obtained by a short course and accompanied by an impartial and secret assessment. "Could not some university organize a staff course in higher administration, to be supervised by retired vice-chancellors and held at some centre for research which could be furnished with all the information available about all the universities of the world ?" The course would be held in the vacation and designed for those who had been in charge of departments and for whom higher responsibilities might be predicted. After some experience of the course, universities might limit their higher offices to those who had gained this qualification.

For a scheme of this sort one strong argument from army experience is worth noting. Such faults as were apparent in the British generals of the Second World War were seldom or never attributed to lack of brains. There were failures in leadership ; but the staff college system prevented any repetition of Balaclava. The same cannot be said of the university world. Such a staff college would provide a ready-made group of advisers to which a university might turn for guidance.

There are some arguments on the other side. Some of these will derive from that strange reluctance which academic persons reveal when invited to apply scientific method to university affairs. There is no insoluble problem in academic administration so long as the university is kept down to a reasonable size. But there are problems which remain unsolved merely because those immediately concerned lack the ability to find a solution and because they fail to apply the sort of method which they would apply without hesitation to matters more obviously scientific. In this matter of using a methodical approach to a political problem, the universities have not only a duty to themselves but also a further duty, by way of example, to the world.

THE NUFFIELD FOUNDATION

THE fourteenth annual report of the Nuffield Foundation (pp. xii+201. London : The Nuffield Foundation, 1959), covering the year ended March 31, 1959, records for the first time expenditure on grants exceeding £1 million, and of this £1,137,900, roughly a quarter was spent in the Commonwealth, mostly in Africa, and the rest at home on scientific, medical and social projects and on fellowships. With regard to the last it may be noted that since 1946, 357 travelling fellowships have been awarded, and from the beginning the Foundation has made a special point of enabling the married overseas fellow to be accompanied by his wife and for its married Fellows has provided a small colony of furnished flats in Prince Albert Road, London. Travel grants during the year have covered the visit to England of Mr. R. C. Robinson, a neurosurgeon from the University of Otago Medical School ; a visit to Dr. D. G. Abraham, Ibadan, by Dr. Wallace Brigden to study a puzzling heart condition ; a lecture tour in Canada by Sir Christopher Ingold, arranged by the Chemical Institute of Canada ; and a study visit on Commonwealth constitutional law by Prof. C. H. Alexandrowicz, of the University of Madras. Three medical fellowships, three fellowships and four scholarships in dentistry were awarded during the year, but the scheme for sociological scholarships and bursaries was discontinued and a new scheme of senior sociological scholarships initiated experimentally for three years. Only one travelling fellowship for Home Civil servants was awarded, but eight travelling fellowships were awarded in medicine, twelve in natural science, and nine in humanities and social science, while twenty-three awards were made under the Royal Society–Nuffield Foundation Commonwealth bursaries scheme.

In this report the Foundation, welcoming the new emphasis placed by the Government on the teaching of technology, records that, after considering how best it could help without trespassing on public responsibilities, the Foundation has decided that its chief interest should be in the technological colleges providing advanced courses. These colleges have a different purpose from the technological departments of universities, deriving from the belief that some students mature better if their theoretical training is more closely interwoven with industrial practice than is usual or desirable for university students. It considers that all such colleges need to establish a fruitful partnership with the industries with which they are most intimately associated, and while refraining from adopting a detailed programme of schemes for sandwich courses, for example, for which it would be willing to provide grant-aid, has indicated its interest in experimental schemes designed to achieve closer unity between the colleges and industry and in schemes which would benefit technological industry

generally. As regards support for research at Commonwealth universities and institutions of like standing, the emphasis during the year has been on Africa.

In science, three grants of £50,000 were made during the year: one to the University of Edinburgh for fundamental research under Profs. M. Swann and C. H. Waddington on fibroblasts which may throw some light on the causes of rheumatism; another to the British Museum (Natural History) for the purchase of about half the collection of meteorites formed by Mr. H. H. Niniger, of Arizona; and the third, over five years, to the University of Cambridge for the development by Dr. M. V. Wilkes, of the Mathematical Laboratory, of a much faster digital computer, *Edsac* 3, capable of solving partial differential equations. Among other grants may be noted a further £9,000 over three years in support of the study of protein metabolism and particularly connective tissue under Prof. A. Neuberger at St. Mary's Hospital Medical School; £4,350 over three years for an investigation by Dr. I. Rorison, of the University of Nottingham School of Agriculture, of the effects of aluminium on plant growth; £11,000 over three years for the biochemical and physiological investigation of cell division and expansion in the meristems of the shoot and root at the University of Edinburgh; and a further £5,250 over three years to enable Dr. A. G. M. Weddell to continue his studies at the University of Oxford on the mechanisms of common sensation; and £1,690 a year for three years to King's College, London, for an investigation of the cell cortex and cell movement under Prof. J. F. Danielli.

In technology a research unit for psychological and social research on problems of technical education and human factors in industry has been established at Brunel College of Technology, Acton, with the help of a Foundation grant of £15,000, and £12,000 over five years was also offered to the University of Birmingham to establish a research unit in the Institute of Education under Prof. E. A. Peel to study and advise on the placing, counselling and teaching of part-time technical college students.

Apart from the grants to the University of Edinburgh for work on fibroblasts and the nature of the enzyme systems involved, all other new grants for research in rheumatism have been made to departments or projects already supported by the Foundation. These included £3,000 over three years for an electron microscope study at the Rheumatism Research Centre, University of Manchester, of the fine structure of the fibroblast in different phases of its metabolic activity, and £4,100 to the University of Sheffield for work under Dr. H. F. West in the Sheffield Centre for the Investigation and Treatment of Rheumatic Diseases. Other grants for medical research have included £25,000 over five years for a metabolic research unit at the Institute of Urology, University of London, and £20,000 towards the capital expenditure at Banstead Place and Dorincourt of two units for the rehabilitation of young chronic sick, chiefly for building and equipping a physiotherapy unit at Banstead Place.

Grants in the United Kingdom for social research and experiment in education include one of £7,500 over three years to the School of Architecture, University of Cambridge, for studies of urban reconstruction, particularly of urban housing in relation to daylight, sunlight and open space, including the provision of special apparatus, such as an artificial sky and the necessary measuring instruments for the study of day-lighting. A grant of £4,000 for two years was made to the Christian Frontier Council in aid of a study of the notion of equality in modern society with special reference to its implications for politics, industry and education, and for the pursuit of the ideals of excellence; one of £2,200 to the University College of North Staffordshire to enable a study of Parliament on the lines of a pilot study completed for 1955–58 to be made for the period 1945–55; a grant of £1,250 to the Institute of Race Relations, London, for a study of the integration of alien or minority groups of workers (chiefly West Indians and Poles) in an industrial situation in the London area; one of £3,700 to the School of Social Studies, University of Sheffield, for an inquiry by Mr. R. R. Kelsall into wastage, after training, among women teachers in England and Wales. A grant of £2,000 was made to the University of Manchester to enable Prof. S. Devons to try out his scheme of using honours graduates in physics part-time on research at a university and part-time on school teaching to relieve the shortage of first-class teachers of science in grammar schools, and £8,500 was offered to the Scottish Council for Research in Education to permit a follow-up survey between eleven and eighteen of the mental survey of the whole eleven-year age-group of more than 70,000 children in Scotland made in 1947, while up to £3,500 was offered to initiate "Youth Ventures", a non-profit making company intended to provide an unconventional youth service to fill in the gaps until the 'new towns' are more fully developed commercially.

In the field of research in ageing and care of old people, £6,000 was provided to the University of Cambridge for further studies of the economic position, and a grant of £3,700 to the University of Bristol for studies of the employment of older workers was renewed for a sixth and final year. The University of Birmingham received another £6,500 for Prof. P. L. Krohn's unit studying the ageing ovary, and the University of Leeds £6,000 for a further year's study by Dr. D. A. Hall's team of the changes taking place in connective tissue and the processes concerned in the degradation of such tissue during ageing, while £4,500 over the next three years was provided for work on physiological and pathological changes in the aged under Dr. E. Woodford-Williams at the Sunderland General Hospital.

Among grants for the Commonwealth Overseas during the year are £3,000 over two years for a study of the normal lymphatic system in typical primates, and £3,320 over two years for studies on the helminthic fauna of freshwater fish in Central Africa at the University College of Rhodesia and Nyasaland; up to £50,000 including a capital grant up to £30,000 over five years for an experiment in rural education by the Ministry of Health, Kenya; £5,000 to University College, Ibadan, in support of epidemiological field work in Nigeria; £3,000 as an emergency measure to extend the life of the Medical Research Unit, University of Natal; £20,000 over two years to the East African Agriculture and Forestry Research Organization for grassland reclamation in the Ngorongoro Crater highlands; a further £5,000 to the Gayaza Girls' High School for its study of farming and a balanced diet; a further £5,750 to the University College of East Africa for its swamp research; £13,850 for the study of urbanization in the Sierra Leone Protectorate being undertaken by the University of Edinburgh under Dr. K. L. Little; £12,000

for five years to the radio education unit of the University College of the West Indies; £20,000 over three years on a tapering basis to the Government Central Pedalogical Institute, Allahabad, for teaching English; up to £4,000 to the University of Hong Kong to enable Prof. J. E. Driver to purchase equipment for his Department of Chemistry, and £3,600 over three years to the Institute of Child Health, Calcutta, for studies of the pattern of disease in a sample group of children.

PROGRESS IN FOOD INVESTIGATION

FOR many years the annual report published by the Department of Scientific and Industrial Research under the title "Food Investigation" has reviewed the work of the Food Investigation Board and of three research institutes, namely, the Low Temperature Research Station at Cambridge, the Ditton Laboratory, Maidstone (with its associated unit at Covent Garden), and the Torry Research Station, Aberdeen.

In 1958 the Council of the Department of Scientific and Industrial Research decided on a series of administrative changes: (1) to disband the Food Investigation Board; (2) to retain the Torry Station as an independent establishment of the Department; (3) to transfer the other two institutes to the Agricultural Research Council. This latter change was brought about "because the two Councils believe that it is in the national interest to bring all Government research on food, other than fish, under one organization, the aim being to make the research more effective". From now onwards a report on the Torry Research Station will be published annually by the Department of Scientific and Industrial Research; the report, "Food Investigation 1958"*, is limited to the Cambridge and Ditton Laboratories and will be the last to appear under the auspices of the Department.

The Cambridge and Ditton Laboratories are complementary to one another: at Ditton the emphasis is on problems associated with fruits and vegetables; at Cambridge, although important work is being carried out on plant tissues and products, the major stress has been on animal products—meat (including bacon and poultry), eggs and animal fats. At both centres there have been fundamental investigations in biochemistry, biophysics and microbiology, and the report illustrates how difficult it is (in any event in the field of food technology) to draw a distinction between pure and applied research; the examination of the factors governing the quality of meat, eggs or fruit has stimulated basic work on the chemical composition of these materials and on the nature of the enzymes present; in turn, the newer information obtained has helped in the solution of practical problems of food storage and preservation.

The Cambridge section of the report includes summaries of investigations on three recent developments in food technology, namely, the administration of œstrogenic hormones to young animals (to increase the rate of gain of weight and the efficiency of food conversion), the use of antibiotics in poultry storage and of radiations in meat preservation. Details are given also of recent research on the phenolic constituents of plants; these compounds—ranging from relatively simple molecules to complex polymers—are now known to play a significant part in the control of colour, flavour and texture in a wide variety of foods.

The Ditton Laboratory records the increased commercial use of the hydrocooling process for vegetables such as watercress and lettuce; controlled experiments have shown that vegetables subjected to this process should arrive fresher on the market and retain their quality for a longer period. Another important line of research is concerned with the storage of potatoes in bulk using a new method of suppressing sprout growth—the introduction of nonyl alcohol vapour into the ventilation system of the store. An account is given also of various lines of research in plant physiology and biochemistry, and in refrigeration and other aspects of 'biological engineering'.

The food industries in the United Kingdom owe a debt of gratitude to the Department of Scientific and Industrial Research for sponsorship of research over some decades; the annual reports of the Food Investigation Board and other publications from the individual laboratories have played an indispensable part both in stimulating industrial research and in placing food storage and processing in the United Kingdom on sound scientific foundations.

FRANCIS AYLWARD

* "Food Investigation 1958." Pp. vi+32. (London: H.M. Stationery Office, 1959.) 3s.

THE HOVERCRAFT

A DEVICE for forcing out compressed air between a ship's surface and the water was patented in 1883. It did not work. Many similar fruitless attempts were made in the next sixty years until, in 1953, Mr. C. S. Cockerell began experimenting with air lubrication. His success is now universally recognized and the first hovercraft has begun the hard task of practical development, foreshadowing a new contender in the field of maritime transport.

Cockerell began as had De Laval, by attempting to contain a sheet of air between the surface of a vessel, in this case a planing craft, and the sea. He found that the reduction in drag was offset by the hull modifications needed to supply the air. He then attempted to contain the air lubricating sheet between vertical plates but these plates increased the skin friction drag. The answer was to replace the solid containing plates by curtains of air and so the hovercraft was evolved.

The experimental version now flying, the 'SR-NI', is an oval dish on top of which are mounted the propulsion and control systems, and the air compressor. The compressor is a 7-ft. diameter ducted fan powered by a 435-h.p. internal combustion aero engine. Two-thirds of the compressed air is fed to two concentric annular slots near the periphery of the

under surface of the oval disk. The slots are shaped so that two continuous jet sheets are directed downwards and slightly inwards. When the engine is started the jets first build up the air cushion under the disk and then maintain the pressure difference between the cushion and the atmosphere. The pressure difference deflects the air curtains and they spill outwards at the ground or water surface (the pressure difference times the height above the ground must equal the radial rate of change of momentum). Two curtains are used for stability reasons. A third of the compressed air is bled off into two parallel propulsion ducts of rectangular cross-section which lie along and above the flat sides of the oval disk. Vanes at each end of each duct provide the necessary longitudinal and lateral control.

Because the weight that can be supported is dependent on the disk area while the power required is proportional to the peripheral length, the economy of a hovercraft should improve with size. A reasonably flat operational surface is required and early applications may well be over surfaces which existing vehicles find difficult, for example, marsh, snow, sand, ice and shallow rivers. Serious consideration has been given to a 400-ton car ferry capable of carrying eight hundred passengers and eighty cars across the channel at a speed of 90 knots. A careful detailed study of hovercraft economics suggests a cost of between 8 and 18 pence per (payload) ton (nautical) mile in the speed-range 70–170 knots. Both cost and speed lie somewhere between the current values for ships and commercial aircraft. If the hovercraft can prove its worth with modest payloads over short distances, then serious thought could be given to developing a trans-ocean hovercraft capable of crossing the Atlantic in 24 hr. This new competitor could not appear within the next ten years, however, and one wonders what the level of ship and particularly aircraft fares will be by then. Nevertheless, the hovercraft has an extremely interesting future and one can only congratulate the inventor and the development engineers on their achievement and remark that this is one enterprise where the Old World leads the New.

These remarks are based on a paper "The Hovercraft—a New Concept in Maritime Transport", by P. R. Crewe and W. J. Eggington, read before the Royal Institution of Naval Architects on November 19, 1959.

J. L. Stollery

NEW DELHI SYMPOSIUM ON ALGAE

Possibly because algae are usually inconspicuous in the temperate regions in which most microbiologists receive their training, the dominant role which these organisms may have in soil and water in other climates is apt to be overlooked. In India, although much excellent work has been done on algae, this has largely followed morphological and systematic lines, and relatively little attention has been paid to their activities and possible economic importance. The symposium on "Algology", held in the Indian Agricultural Research Institute, New Delhi, during December 7–11, under the auspices of Unesco and the Indian Council of Agricultural Research, had the object of stimulating investigations on the more practical aspects of the subject. It was opened by Mr. S. K. Patil, Minister for Food and Agriculture, and was attended by experts from Japan, England, the Federal Republic of Germany, and Israel, as well as by delegates from all parts of India.

An introductory address by Dr. M. S. Randhawa, chairman of the organizing committee, outlined the development of algal studies and lines of research which may be of practical importance. The principal uses to which these organisms may be put are mentioned below, but it may be noted that in the tropics algal growth often needs controlling rather than encouraging—a matter which was not considered in any of the papers which followed. The growth of blue-green algae, which generally occurs on buildings during wet weather, not only renders them unsightly but also corrodes the stone-work and presents a problem of major economic importance. Algal growth may also interfere in the manufacture of salt, it may block canals and irrigation channels, and pollute fresh water.

The papers presented covered nearly every aspect of modern knowledge of the algae. Classical morphology was represented by papers on the comparative morphology of the Charophytes, by V. S. Sundaralingam, and on reproduction in red algae, by M. S. Balakrishnan, and by a lecture by M. O. P. Iyengar, describing some of the many interesting species which have been discovered in India. Recent advances in the study of the submicroscopic morphology of algae were reviewed by T. V. Desikachary, who also discussed the structure of diatom frustules in the light of his own electron microscope investigations. Papers on algal cytology, which is especially important for the understanding of life-cycles and for taxonomy, were given by C. S. P. Rao, Y. S. R. K. Sarma and Y. B. K. Chowdary.

One of the most important of recent advances in algal physiology has been the introduction by H. Tamiya and his associates of the technique of synchronous culture as a means of studying the metabolic changes occurring during the life-cycle. A paper by H. Tamiya summarized the main results obtained and dealt particularly with the role of sulphur in the processes of cell maturation and division. Studies on the mineral nutrition of a *Selenastrum* were described by K. V. Natarajan. In a lecture on the extracellular products of algae, G. E. Fogg presented evidence showing that the liberation of these is widespread and that enzymes are included among the very varied kinds of substances involved. These extracellular substances may be of ecological importance in various ways, as, for example, by forming chemical complexes with inorganic ions. The kinetics of the production of extracellular nitrogenous substances by a nitrogen-fixing *Nostoc* isolated from the root nodules of *Trifolium alexandrinum* was discussed by G. S. Venkataraman. In another paper on nitrogen fixation, G. E. Fogg emphasized the close intermeshing of this process with photosynthesis which occurs in blue-green algae. He also reported nitrogen fixation by *Chlorogloea fritschii*, evidently the first unicellular member of the group in which this property has been

demonstrated unequivocally. A paper by A. Watanabe dealt with the importance of blue-green algae in rice-fields. Nitrogen-fixing blue-green algae are found almost ubiquitously throughout tropical. sub-tropical and temperate regions and evidently contribute materially to the fertility of many soils. It has been found possible to grow *Tolypothrix tenuis*, a particularly vigorous nitrogen-fixing species, on the large scale and to preserve it as a culture on porous gravel. Inoculation of paddy fields with this has resulted in appreciable and long-continued improvements in yields of rice.

H. Tamiya, H. von Witsch and A. M. Mayer each considered various aspects of the mass culture of microscopic algae such as *Chlorella* for use as animal or human food. The average yields of *Chlorella* grown on the large scale correspond to the production of 14,000 lb. of protein per acre per year, whereas the corresponding figures are 600 lb. for grass, 420 lb. for peanuts, 90 lb. for milk and 54 lb. for meat. The results of feeding tests demonstrate that the alga is of high nutritive value. Convincing evidence of the feasibility of large-scale culture was given in a film of the work of the Japan Micro-algae Research Institute. This has culture ponds of about an acre in total area producing 12 tons dry weight of *Chlorella* a year, which is largely used as a source of growth factors for the commercial culture of *Lactobacillus*. As a means of producing material which can be used directly in human or animal nutrition the process is not yet an economic one; the estimated cost of decolorized algal powder is 1·12 dollars per lb.—considerably more than that of cheap protein sources such as soy beans (0·06 dollar per lb.) or fish meal (0·13 dollar per lb.) but a little less than that of skim milk powder (1·20 dollars per lb.). In arid regions this high cost may be offset by the high degree of water conservation which is possible with algal cultures as compared with conventional crops, but for more general use cheaper methods are essential. To suit Indian conditions, it would be desirable to simplify the process so that it might be operated by peasants, and to this end M. O. P. Iyengar suggested that an examination should be made of algae known to grow prolifically in Indian fresh waters and which might be harvested easily.

The cost of algal culture can be minimized by combining it with the photosynthetic treatment of sewage and organic wastes by the oxidation pond method. A paper submitted from the Sanitary Engineering Laboratory of the University of California gave a résumé of this process and reported that algae harvested from oxidation ponds by alum flocculation proved satisfactory as a protein source in the diet of lambs. Field studies carried out at the Engineering College, Guindy (Madras), on the use of algae in sewage disposal, described by A. Raman, indicate that the method can be used under Indian conditions.

In a general lecture on the use of algae as food, H. Tamiya, besides describing the possibilities of *Chlorella*, gave an account of the use of seaweeds for this purpose in Japan. Among these, the most popular is *Porphyra tenera*, which is cultivated in shallow seas to the extent of 4,000–5,000 tons dry weight per annum and which can command prices of up to 8.00 dollars per lb. The amounts of marine algae available around India are relatively small, but a paper by F. Thivy showed that there are, nevertheless, possibilities for their use as food and described cottage industry methods for the preparation of seaweed meal and agar. The ecology and distribution of Indian marine algae were considered in papers by J. N. Misra and K. S. Srinivasan.

Phytoplankton ecology was discussed in four papers. V. P. Singh described detailed observations made over a year on two lakes in Uttar Pradesh, the plankton of which is dominated by *Microcystis*. M. T. Philipose and S. V. Ganapathi gave more general descriptions of Indian freshwater phytoplankton in relation to fisheries and water supply. The marine phytoplankton of the west coast of India was considered in special relation to fisheries by R. Subrahmanyan. The indications are that the phytoplankton production could support a considerably higher rate of fishing than it does at present.

At the final session a number of recommendations were approved for the extension of algal research in India on a broad front with the ultimate object of making more practical use of these organisms.

G. E. Fogg

EROSION OF METEORITES IN SPACE AND THE DENSITY OF INTERPLANETARY GAS

By Dr. L. REIFFEL

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THE etching rate, due to interaction with interplanetary gas, for the Sikhote-Alin meteorite fall has been estimated[1] as $\lesssim 2 \times 10^{-7}$ cm./yr. from an exposure life-time of 5×10^8 years, as determined by argon-38/argon-39 age measurements. From this result, Whipple[2] has suggested that the maximum possible proton density of interplanetary space in the vicinity of the Earth is as shown in column 3 of Table 1. Whipple assumes that meteoritic etching is due solely to sputtering from solar protons and uses normal incidence sputtering yields for protons on iron as follows: 0·5 atom/proton for 10,000 eV. or more, 0·2 at 500 eV. and ~0·02 at 100 eV. In the actual calculation, these yields are doubled to account, approximately, for the greater yields which are manifest at smaller angles of incidence.

The purpose of this article is to point out that solar protons are probably not the major factor in determining etching rates from sputtering by gas clouds ejected from the Sun. Calculated upper limits on interplanetary gas densities and erosion rates near the Earth may be changed appreciably as a consequence of including sputtering by elements heavier than hydrogen, and are uncertain for other reasons as discussed below.

In spite of the lower abundance of helium relative to hydrogen, the greater momentum transfer and sputtering efficiency for this ion dominate the meteoritic sputtering process under the assumptions: (1) the ejected solar gas has a chemical composition approximately duplicating that of the solar corona; (2) significant fractionation of helium does not occur in the solar acceleration and escape processes. Since solar streams appear to be "segments of the corona, blown out bodily"[3], these assumptions do not appear unreasonable. In this connexion, it should be noted that Shklovskii's discussion[4] of sources for helium in the terrestrial atmosphere gives strong support to the view that the stream composition is similar to that of the Sun itself. If helium ions travel along in the ejected clouds with the same velocities (column 2 of Table 1) as the stream protons, they will, of course, have four times as much energy, and their relative flux will be the same as their relative abundance. The atomic abundances of hydrogen and helium in the solar atmosphere are difficult to determine. Neven and DeJager[5] suggest an hydrogen/helium ratio (δ) of 20/1 while Unsöld[6] finds 5/1. Recently, Aller[6] has given 7/1 as the best value currently available.

Sputtering yields do not appear sensitive to energy for either hydrogen or helium ions in the range 1–10 keV. according to recent measurements by Grønlund and Moore[7]. These data, obtained with analysed beams known to contain only ions, refer to silver targets, but iron is expected to behave in a very similar manner. The ratio of helium ion sputtering efficiency to hydrogen sputtering efficiency (β) is roughly fourteen over the entire range studied. We shall compare hydrogen and helium yields at the same energy, thereby implicitly allowing for somewhat reduced acceleration efficiency for the heavier ions, and shall assume that the relative sputtering efficiency will not change radically when yields are integrated over all angles of incidence. Using the appropriate abundance and allowing for differences in sputtering efficiency, it is seen that the helium erosion is comparable to, and probably exceeds, that due to hydrogen, implying that Whipple's upper limit to the solar proton density can be markedly reduced if the effect of helium is included. Specifically, if N'_p is the proton density calculated by Whipple, one may write for the corrected proton density N_p:

$$N_p = N'_p\left(1 + \frac{\beta}{\delta}\right)^{-1}$$

Here I have retained Whipple's assumptions in allowing for angular dependence of yield (a factor of 2), absolute sputtering yield for protons (as listed earlier) and scaling from fluxes at the meteorite orbit to those near the Earth (a factor of 3/2). Results calculated from this expression, using $\delta = 7$ and $\beta = 14$, are shown in column 4 of Table 1. Including the high Z elements other than helium would reduce N_p still further, but unless their sputtering efficiency is surprisingly high, the change would not be large.

The figures of column 4 are low enough (especially at the higher velocities which, on the basis of other evidence, seem to be most probable) that one becomes concerned about maintaining explanations of a variety of other astrophysical and geophysical phenomena, which, in the past, have led to 'conventional' solar gas densities (at 1 A.U.) between 10^3 and 10^5 protons/cm.^{3} under quiet conditions with estimated velocities varying upward from 500 km./sec.

Table 1. Proton Densities near Earth as inferred from Meteoritic Erosion Rates

(1) Proton energy eV.	(2) Proton velocity km./sec.	(3) Number* per cm.^{3}	(4) Number† per cm.^{3}	(5) Number‡ per cm.^{3}
500	310	290	95	1,000–1,500§
1,000	440	140	45	650
5,000	980	40	13	186

* After Whipple.
† Corrected for sputtering by helium.
‡ Corrected for sputtering by helium and using analysed beam sputtering yields.
§ Rough estimate using $\beta = 14$, which may not hold for this energy.

Furthermore, Russian observations[8], while unfortunately not providing velocity information, have recently yielded measured densities which also lie between 10^3 and 10^5 protons/cm.^{3}. Recent laboratory sputtering results using analysed beams are of especial interest in this situation since they are markedly lower than the figures employed by Whipple which were obtained by Wehner using gas-discharge techniques. If one assumes that absolute yields of the order of those observed by Grønlund and Moore for protons on silver (0·035 at 5,000 eV.) are also appropriate for iron, the allowable proton densities rise markedly as shown in column 5 and come into conformity with estimates from phenomena other than meteoritic erosion. It may be that resolution of the somewhat conflicting requirements on fast (for example, >500 km./sec.) solar stream densities implied by meteoritic life-times and other types of observations lies, at least in part, in the reduced sputtering by beams containing only atomic ions. Completely ionized beams would be the rule in space, but perhaps have not been attained in most laboratory sputtering experiments. On the other hand, if the average solar proton velocities prove to be 300 km./sec. or less, one need not appeal to explanations of the sort suggested here.

Finally, it should be noted that any of the results given in Table 1 must be viewed with caution for a number of reasons beyond those already discussed:

(1) The meteoritic etching rate employed at the outset may refer to heliocentric orbits at relatively high angles to the ecliptic and therefore may be anomalously low. Many meteors have been observed in 60 degree orbits. It appears that the solar corona, and probably solar streams, are markedly more dense in the ecliptic[9]. Determination, from fall data, of the orbits of meteorites is difficult and generally unreliable[10], hence study of a statistically significant number of meteorites would be highly desirable before reaching final conclusions.

(2) Energy transfer from the ions to the electrons in the region of solar stream instability may occur[11] and might be accompanied by a spreading of the solar streams away from the ecliptic, resulting in reduced particle densities. If the admittedly speculative transfer process is effective, some shift in the particle energy spectrum would be produced in the region beyond 1–2 A.U. Thus, in the asteroid belt, where the meteorite may have spent the greater part of its exposure life-time, it would be bombarded by an electron spectrum of higher energy and a proton spectrum of lower energy than that characteristic of the inner solar system. Decreased sputtering would result, so the relative gas densities and erosion-rates near the Earth would increase.

(3) If the meteorite originated in the asteroid belt and was deflected into an Earth-capture orbit in a

collision, it may have spent some of its life well beyond 2 A.U., since most asteroids have mean distances from the Sun lying between 2·0 and 3·5 A.U., but are found over a much wider range. Other than by statistical arguments, it does not seem possible to state definitely that the meteorite spent most of its life in an unperturbed Earth-capture orbit with orbital elements near those of the Earth itself. Thus, a square-law dependence of solar stream densities could introduce appreciable uncertainty in scaling from the meteorite orbit to a circular orbit of 1 A.U. radius.

Direct measurement of both erosion rates on known surfaces, as well as particle densities and velocities in space near the Earth, would obviously be of great value, as would collection and analysis of additional data on meteoritic etching rates. Experiments are planned in various laboratories to make direct observations on interplanetary proton densities. In the present context, it would seem desirable to attempt simultaneous measurements on helium.

[1] Whipple, F. L., and Fireman, E. L., *Nature*, **183**, 1315 (1959).
[2] Whipple, F. L., *J. Geophys. Res.*, **64**, 1655 (1959).
[3] Keipenheuer, K. O., "The Sun", ed. G. P. Kuiper, **1**, 449 (University of Chicago Press, 1953).
[4] Shklovskii, I. S., *Soviet Astronomy*, **2**, 516 (1958).
[5] Quoted by Aller, L. H., in "Handbuch der Physik, Astrophysics II : Stellar Structure", **52**, 344 (Springer Verlag, Berlin, 1958).
[6] Aller, L. H. (private communication, January 5, 1960).
[7] Grønlund, F., and Moore, W. J., *Science*, **30**, 1417 (1959); *J. Chem. Phys.* (in the press). I am indebted to Dr. Moore for communication of his results prior to publication, as well as helpful comments on the relative behaviour of iron and silver.
[8] Sedov, L. I., and Krassovsky, V. I. (private communication, November 20, 1959).
[9] See discussion, Symposium on Exploration of Space, *J. Geophys. Res.*, **64**, 1693 (1959).
[10] Urey, H. C., see discussion, Symposium on Exploration of Space, *J. Geophys. Res.*, **64**, 1733 (1959).
[11] Reiffel, L., *Nature*, [**185**, 229 (1960)].

DIETARY PHYTATE AS A POSSIBLE CAUSE OF MAGNESIUM DEFICIENCY

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WE have recently made a study in the rat of the effects of dietary phytate upon intestinal phytase and bone calcification. A short account of some of the results has been published[1].

During the course of these observations, we found that rats given sodium phytate in purified diets became severely ill. We believe that this was due to a conditioned deficiency of magnesium, brought about by the soluble phytate combining with the dietary magnesium, which consequently becomes insoluble and unavailable.

The basal purified diet was made up of sucrose 60, low-vitamin casein 20, arachis oil 15, salt mixture 5. Additions of sodium phytate (Ciba) were made at the expense of sucrose. Vitamin supplements were given separately. Most of the experiments were carried out with a strain of albino rats; in a few experiments we used hooded rats of the Lister strain, and obtained similar results, though this strain appeared somewhat less sensitive to phytate. This recalls the observation on chronic magnesium deficiency in two similar strains, described by Watchorn and McCance[2].

We observed the signs of phytate intoxication in albino rats with levels ranging from 1 to 10 per cent sodium phytate in the diet. The animals were put on the experimental diet at 24 days, when they weighed about 45 gm. At a level of 10 per cent phytate, the rats developed severe diarrhœa within 24 hr. and rapidly lost weight. Within a week, seven out of the eight rats in this group had died, having lost about 10 gm. body-weight. The last rat survived for four weeks, but at the end of that time weighed 64 gm., about one half the weight of the control animals receiving no phytate.

With 7½ per cent sodium phytate, severe diarrhœa again developed in the first day, but subsided during the second week. The rats lost weight during the first week, then began to put it on again. They were nevertheless much thinner than normal, with a coarse staring coat and bald patches. The nose was swollen and covered with a dark red bloody exudate. The abdominal wall was hard, and there was often priapism. The animals were very irritable and difficult to handle. They died after about 18 days on the experiment, weighing about 80 gm., with evidence of having passed blood rectally.

With 5 per cent sodium phytate, diarrhœa also occurred, but less severely and rather later. The rats gained weight slowly after the first week. They also developed swollen noses with exudate. One out of the four animals had a convulsive fit in the third week, and showed paralysis from the waist downwards. It lay on its side, breathed very rapidly and died within 24 hr. The others died after about 25 days, weighing about 105 gm. Hooded rats showed an exactly similar response to 5 per cent phytate.

With 2½ per cent sodium phytate, there was only slight diarrhœa in the first week. Bloody exudates again appeared on the noses of the rats. Two out of four rats had convulsive fits and developed paralysis a few days before death. All of them died after about 50 days, weighing about 150 gm.

With 1 per cent sodium phytate, there was no diarrhœa. The bloody nasal exudate appeared in two out of four rats. Growth was better, though still less than that of the control animals receiving no phytate. One rat was found dead after five weeks, and one after eight weeks; there was no sign of convulsions or of paralysis. The other two rats survived at least nine weeks. On this level of 1 per cent sodium phytate, 16 hooded rats showed no signs other than diminished growth.

At first, we examined the possibility that the signs were due to deficiency either of calcium or of inositol. Calcium deficiency could arise by the phytate making the dietary calcium unavailable. We did not think that calcium deficiency was very likely; while it might perhaps account for the convulsions and paralysis, the other signs have not been described in calcium deficiency. Moreover, the toxicity produced by phytate did not occur with dietary citrate, which

like phytate would have rendered calcium unavailable. Nevertheless, we tried to cure or to prevent phytate toxicity by the addition of calcium to diets containing phytate, or by the parenteral injection of calcium gluco-galacto-gluconate ('calcium Sandoz'). We found no difference in the signs produced by phytate when the rats were given calcium.

Inositol deficiency could arise by competitive inhibition, since phytate is inositol hexaphosphate. Moreover, the signs we observed were similar to those which had been reported for inositol deficiency in rats. We therefore gave 50 mgm. inositol daily, in addition to the 1·1 mgm. contained in the routine vitamin supplements, to half of a number of rats receiving varying levels of phytate from the age of 24 days. This made no difference in growth or in the development of signs of phytate intoxication.

We now examined the possibility that phytate intoxication was due to magnesium deficiency. We recalled that Kruse, Orent and McCollum[3] and Watchorn and McCance[2] had reported signs of magnesium deficiency in rats, which resembled those in our rats showing phytate intoxication. We therefore gave levels of phytate up to 7½ per cent to groups of eight animals; four on each level had in addition 10 gm. magnesium sulphate to 100 gm. diet. The additions were made after the animals had been on the control or phytate diets for three weeks, or (with 7½ per cent phytate) two weeks. The general effect of the magnesium supplements was to improve the appearance of the animals; for example, the nasal exudates were much reduced, and there were no bald patches. There was no effect on the growth of the animals receiving 1 per cent phytate, but there was improved growth and longer survival in those receiving 2½, 5 or 7½ per cent phytate. In the 5 per cent group, for example, the rats receiving magnesium gained on average 20 gm. in weight in the week following the addition, whereas those not receiving the magnesium gained 8 gm.; the rats receiving magnesium survived at least 56 days, whereas those not receiving it died after an average of 41 days.

In this experiment, there were two animals which showed exceptional responses. One was receiving a diet with 1 per cent sodium phytate; it was also given magnesium supplements, but in spite of this it died in convulsions. The second animal was receiving a diet with 7½ per cent phytate; it was not given magnesium supplements, yet nevertheless it showed no signs of phytate intoxication throughout the experiments. These great variations between individuals in the development of signs of hypomagnesæmia are well known in cattle (Allcroft, R., personal communication). Apart from these individuals, however, all the rats receiving 2½ per cent or more of phytate without magnesium were either found dead, or developed fits and died within 24 hr.; all the rats receiving the same diets with magnesium gained weight and survived for the duration of the experiment and showed little if any signs of intoxication.

A second experiment was carried out with five groups, each of eight rats. One group received no phytate, two groups received 5 per cent phytate and two groups 7½ per cent phytate. One group on each level of phytate was given in addition magnesium sulphate at the rate of 10 gm. per 100 gm. diet. The rats on the phytate-free diet, or on the phytate diets with magnesium sulphate, all grew and survived until the experiment was terminated after nine weeks. Those receiving 5 or 7½ per cent phytate with no magnesium grew badly, and died on average at 42 days or 20 days (see also Fig. 1). Although the addition of magnesium greatly improved the condition of the animals receiving phytate, their rate of growth was lower than that of the controls receiving no phytate.

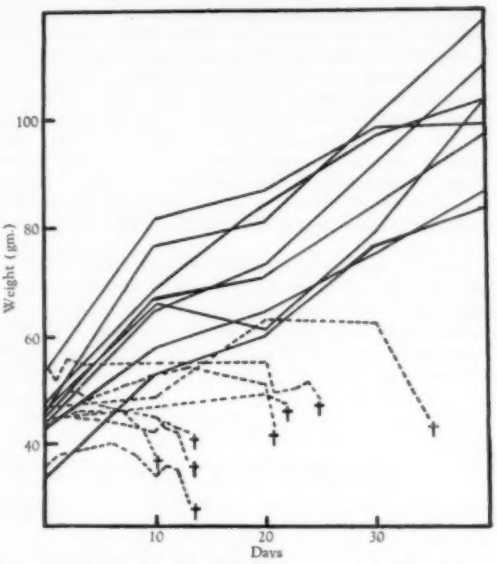

Fig. 1. Effect of addition of magnesium to a diet containing sodium phytate
All rats were given a purified diet with 7·5 per cent sodium phytate from age 24 days. Curves represent weight changes in individual rats. ——, diet with 10 per cent magnesium sulphate; – – –, diet without magnesium sulphate

Since our work was primarily designed to study quite a different problem, we have not yet pursued the many questions which are raised by these results. In particular, we have not examined levels of magnesium in the blood. Nor have we at present facilities for studying the condition in ruminants. We believe, however, that we have reasonable circumstantial evidence that diets which include phytate may cause magnesium deficiency.

We may summarize this evidence as follows: (1) soluble phytate can form insoluble mixed salts of calcium and magnesium; (2) the signs of intoxication shown by rats given diets rich in phytate resemble those described in rats given diets deficient in magnesium; (3) rats show a varying individual susceptibility to phytate intoxication, recalling the known variability of signs of magnesium deficiency in cattle; (4) the signs of phytate intoxication are to a great extent prevented or cured by the administration of magnesium; (5) the doses of magnesium needed to prevent or cure the signs of phytate intoxication in rats are surprisingly large, again recalling the situation in hypomagnesæmia in ruminants.

We suggest, therefore, that a dietary constituent such as phytate, which can render magnesium unavailable, may be a cause of hypomagnesæmia in adult ruminants. It is likely, for example, that this is the explanation of the results of McAleese and Forbes[4], who showed that a diet high in roughage can cause hypomagnesæmia in lambs. The roughage included corn cobs, and corn cobs were also used for bedding. Although we do not know how much phytate was consumed, and how much of it was

soluble phytate, it is likely that the small amount of dietary magnesium was still further reduced by combination with soluble phytate.

Our hypothesis could also explain why the level of serum calcium is often decreased in hypomagnesæmia in adult cattle[5]. Substances such as soluble phytate form insoluble salts with calcium as well as magnesium, so that excess of dietary phytate would lead to decreased absorption of both.

The confirmation of our hypothesis requires much further work. In particular, we need to know the effect of dietary phytate on the magnesium and calcium levels of blood, and whether the pastures which cause hypomagnesæmia in ruminants contain high amounts of phytate or some other constituent which binds the magnesium.

[1] Roberts, A. H., and Yudkin, J., *Proc. Nut. Soc.*, **18**, 27 (1959).
[2] Watchorn, E., and McCance, R. A., *Biochem. J.*, **31**, 1379 (1937).
[3] Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, **961**, 519 (1932).
[4] McAleese, D. M., and Forbes, R. M., *Nature*, **184**, 2025 (1959).
[5] Allcroft, W. M., *Vet. J.*, **103**, 2 (1947).

FLUORESCENCE STUDIES OF CHLOROPLAST DEVELOPMENT IN *EUGLENA*

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EUGLENA should be an excellent organism for the study of control and biosynthesis of a new organelle in response to a highly controllable and defined external stimulus because the chlorophyll-free and presumably chloroplast-less dark-grown organism is believed to develop both rapidly after being placed in the light. An early electron microscope study[1] of the transition seemed to show no detectable chloroplasts in dark-grown cells. As pointed out by Nishimura and Huzisige[2] the results of that work are questionable because Wolken and Palade reported the presence of a very considerable amount of chlorophyll in their dark-grown cells whereas in cells grown for many weeks in the dark there is no trace of chlorophyll[2,3]. Indeed, with so much chlorophyll present, it would be expected that some chloroplast remnants would have been found.

The fact that chlorophyll has a strong red fluorescence suggested to us that it might be possible to follow the developing plastids by direct observation with the light microscope under conditions of dark-field illumination. A similar technique has been used by Strugger[4] and by Heitz and Maly[5] for the chloroplasts of higher plants.

Light from a mercury arc was filtered to pass only 435 mμ; a yellow filter in the eyepiece eliminated interference from the light source. A preliminary look at light-grown cells revealed a spirally arranged set of red-fluorescing structures with the typical disk shape of chloroplasts. A similar look at dark-grown organisms revealed a spirally arranged set of light pink structures scarcely larger than points.

The following technique was developed to immobilize the cells for photography. A 5 per cent solution of polyvinyl alcohol in distilled water is prepared by boiling lightly and letting it stand overnight at room temperature to ensure that the alcohol is fully dissolved. A few drops are placed on a clean glass microscope slide and spread over the entire slide; these dry face up for at least 2 hr. at 37° C. A cover should be placed over the slides to prevent dust from falling on them. We find such slides still usable after as many as 3 days in the incubator. Gelatin and agar were tried and found to have too great fluorescences of their own.

The dried slide is placed in observation position in the fluorescence microscope. A small droplet of the solution to be studied is placed on a clean coverslip which is inverted on to the slide. The droplet spreads and is rapidly absorbed by the alcohol layer, immobilizing the cells. Depending on the stage of chloroplast development, there are 2–10 min. for making pictures before observable deterioration is found. Both 'Ektachrome' and 'Superanschchrome' daylight films have been used, though the former has some advantages for this system because of its greater response in the red.

Euglena gracilis var. *bacillaris* was cultured in Hutner's *p*H 3·5 medium as previously described[6]. At zero time, several ml. of dark-grown logarithmic-phase cells were placed in a test-tube in the light of a daylight fluorescent lamp at room temperature. Samples were studied at various times after the cells were placed in the light. The zero-time samples were taken directly from the culture growing in the dark.

At zero time, the cells usually have one or two pinkish bodies in a line of smaller, paler dots of fluorescence usually running spirally along the organism. The organism itself has a bluish fluorescence; the flagellum is light green. By 15 min., the small dots are visibly enlarged and have become orangish. By 1 hr. the dots are orange-red objects about one-fifth the length of a mature chloroplast. There is some indication that, after this time, some of the increase in size of the plastid may be due to coalescence of several smaller bodies.

By 4 hr., some of the plastids are as much as one-third normal length, and are now quite red. There is an appreciable inhomogeneity of development not only from organism to organism, but also among plastids of the same organism. At about this time, the blue fluorescence has begun to disappear and is essentially gone within another hour or so. Thereafter the plastids develop regularly, on the average, with occasional normal-sized chloroplasts being detectable after about 20 hr. in the light.

There appear to be roughly two types of mature structure. In the majority type there are quite normal-looking plastids with occasional hints of a line of red fluorescence running between some of the plastids. In the minority type the connecting lines are broadened so much as to present a ribbon-like structure with the chloroplasts being detectable only by their being more deeply coloured.

From these observations it may be concluded that there are what must be termed proplastids of an appreciable size (perhaps 1μ) even in the dark-grown organism. It also seems clear that the development involves an actual increase in plastid size as well as in complexity. Thus, it would not be true that the chlorophyll simply vanishes when a green cell is placed in the dark, leaving a structure which can later be refilled whenever chlorophyll synthesis is re-initiated. Preliminary observations with the electron microscope support this conclusion.

The frequent links between chloroplasts remain to be interpreted. Two interpretations may be proposed here. First, the entire chloroplast structure in *Euglena* is to be thought of as a single chloroplast with the normally designated chloroplasts being, in reality, more analogous to the grana of higher plants. Secondly, one might consider that the links are the remnants of connexions between dividing plastids.

Preliminary observations of the reverse processes occurring when light-grown cells are placed in the dark have revealed that the steps are the reverse of the synthetic steps, with, however, a much different time-scale. For example, the blue fluorescence reappears only after more than a day in the dark. The plastids in these organisms remain for many days, being reduced to about one-fifth normal size only after about 2 weeks.

A preliminary survey of our collection of mutants has shown appreciable differences even among different albino mutants. For example, one albino has an overall greenish colour; a second has pale yellow-green plastid-like bodies; and a third has no appreciable fluorescence. A yellow-brown mutant looks normal when grown in the dark, but when grown in the light contains structures similar to those found in normal cells after 10–15 hr. of light.

We have been able to use fluorescence as a criterion of purity of plastids being extracted from organisms at various times after being placed in the light. Also, it now appears likely that the fluorescence may be used as a criterion of functional integrity in such extracts.

Finally, there is one observation that can be made only with the freely moving organisms. The entire chloroplast structure does not move with respect to the cell surface, being apparently fixed in some manner to the cell wall or membrane.

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* National Science Foundation Postdoctoral Fellow (1959–60).
[1] Wolken, J. J., and Palade, G. E., *Ann. N.Y. Acad. Sci.*, **56**, 873 (1953).
[2] Nishimura, M., and Huzisige, H., *J. Biochem.*, **46**, 225 (1959).
[3] Huzisige, H., Terada, T., Nishimura, M., and Uemura, T., *Biol. J. Okayama Univ.*, **3**, 209 (1957).
[4] Strugger, S., *Naturwiss.*, **37**, 166 (1950).
[5] Heitz, E., and Maly, R., *Z. Naturforschung*, **8**, *b*, 243 (1953).
[6] Lyman, H., Epstein, H. T., and Schiff, Jerome A., *J. Protozool.*, **6**, 264 (1959).

INCORPORATION OF ORTHOPHOSPHATE LABELLED WITH PHOSPHORUS-32 INTO CEREBRAL PHOSPHOLIPIDS *IN VIVO*

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ALTHOUGH it was originally considered that the rate of incorporation of radioactive phosphate into brain phospholipids *in vivo* was relatively slow, the work of Dawson and Richter[1] showed that the rate of incorporation of label from the trichloroacetic acid-extractable phosphorus compounds of cerebral tissues into phospholipid phosphorus was relatively high. Ansell and Dohmen[2] showed that the incorporation varied for different phospholipids over short time intervals (0–7 hr.), though the difficulties of interpretation are manifold[3]. Marinetti and co-workers have recently published time curves for the incorporation of phosphorus-32 into some of the phospholipids of tissues other than brain for periods of up to 100 hr. following the subcutaneous injection of labelled orthophosphate into adult rats[4]. We wish to record results showing the course of the incorporation of orthophosphate labelled with phosphorus-32 into brain phospholipids and two probable precursors, phosphorylcholine and phosphorylethanolamine, over a period of seven days; some information on the rate of incorporation of phosphorus-32 into the monoesters of brain *in vivo* has been recorded previously[5,6]. The present communication is prompted by a report by Miani and Bucciante who have carried out a comparable study[7].

Three litters of three-week-old rats were injected intraperitoneally with 4μc. labelled orthophosphate/gm. body-weight (supplied in sterile isotonic solution with carrier phosphate by the Radiochemical Centre, Amersham, Bucks) and killed at intervals. Phosphorylethanolamine and phosphorylcholine were separated from the trichloroacetic acid-soluble fraction as previously described[6]. Phospholipids were extracted from the washed tissue and the specific radioactivities of individual phospholipids determined by the method of Dawson[8] as described previously[9]. The results are recorded in Figs. 1 and 2 in which the specific radioactivities of the various fractions (all corrected to the same radioactive standard) are plotted against time.

The specific radioactivity of the acid-soluble fraction of brain tissue rapidly rose and then stayed relatively constant as has been noted by Dziewiatkowski and Bodian[10]; they found that its rate of decline was much slower than that of the blood. The present results (Fig. 1) indicate that phosphorylethanolamine and phosphorylcholine acquire the same radioactivity as the acid-soluble fraction as a whole within 24 hr. Previous observations[6] have shown that after an exchange period of 3 hr. the specific radioactivity of the phosphorylethanolamine in the brain tissue of young animals is only 70 per cent of that of the total acid-soluble fraction, while that of phosphorylcholine is higher, acquiring radioactive phosphate at a rate greater than the majority of other components, but coming into equilibrium with them after 24 hr. As a result of the second observation it was suggested

that the choline phosphokinase of nervous tissue must be particularly active *in vivo*. After long exchange periods, however, nothing can be deduced about the relative rates of incorporation of phosphorus-32 into these components of the brain acid-soluble fraction (Fig. 1) and it is likely that all its phosphorus-containing components are in isotopic equilibrium (cf. ref. 11). Miani and Bucciante[7] have presented results indicating that, for animals 6 days old, the incorporation of phosphorus-32 into brain phosphorylethanolamine and phosphorylcholine is maximal 40 hr. after injection, but they do not record the values for the specific radioactivity of the total acid-soluble phosphorus.

It can be seen that several of the phospholipids steadily acquired radioactivity over a time period of seven days (Fig. 2) as was originally observed for phospholipid phosphorus as a whole for both young and adult rats by Changus, Chaikoff and Ruben[12] and afterwards for young rats by Davison and Dobbing[13]. Phosphatidyl serine acquired it at the slowest rate and phosphatidyl choline at a faster rate than that of the ethanolamine-containing phospholipids (cf. ref. 2). Loss of radioactivity from rat brain phospholipids as a whole is apparently very slow[13].

It was shown previously[2] that 'diphosphoinositide' (more probably a monophosphoinositide[14]) acquired labelled phosphate faster than any other phospholipid and after a short time interval of 3 hr. the specific radioactivity of its phosphorus was about half that of the acid-soluble phosphorus. Phosphorus exchange was apparently proceeding, in fact, at a rate not very different from that of the phosphorylethanolamine in the acid-soluble fraction. The values in Fig. 2 show that after its initial steep rise the specific radioactivity of the inositol phospholipid remained relatively constant; the scatter of the values is due to the fact that the amount of phosphorus estimated in this instance is very small compared with the radioactivity present. Miani and Bucciante[7] have published curves for the long-term incorporation of phosphorus-32 into brain phospholipids of very young rats (4 days old) and of rats of 100 gm. body-weight. Their values for the young animals indicate a sharp peak for the incorporation of phosphorus-32 into 'diphosphoinositide' about 20 hr. after administration

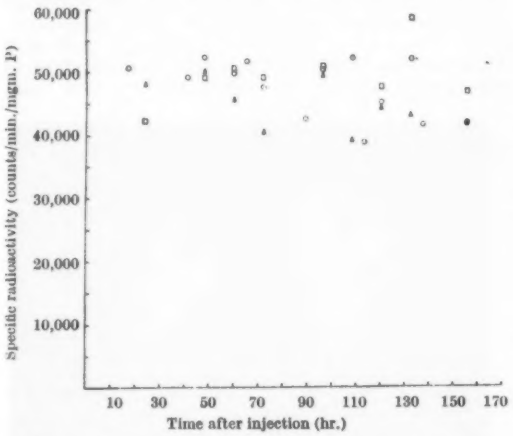

Fig. 1. The specific radioactivity-time scatter diagrams for rat brain acid-soluble phosphorus (○), phosphorylcholine(△) and phosphorylethanolamine (□) after injection of phosphorus-32 labelled orthophosphate when the animals were three weeks old

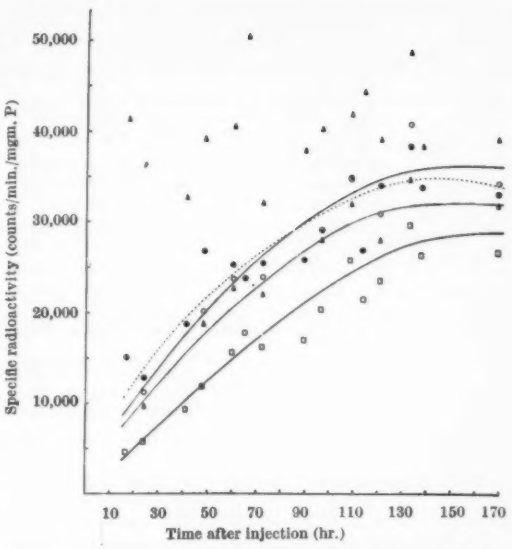

Fig. 2. The specific radioactivity-time curves for rat brain 'diphosphoinositide' (▲), phosphatidic acid (broken line ●), phosphatidyl choline (continuous line ○), phosphatidyl ethanolamine (continuous line △) and phosphatidyl serine (continuous line □)

of the isotope. The specific radioactivity then rapidly falls away; in the older animals the peak occurs about 60 hr. after administration and is flattened; the specific radioactivity then falls away more slowly. It is difficult to correlate these results with our own observations; for the older animals of Miani and Bucciante, incorporation of label into phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine is the reverse of that found by us for animals three weeks old. For the very young animals in the experiments of the Italian workers, the incorporation in the first 15 hr. following injection would appear similar to that observed by Ansell and Dohmen[2]. Afterwards, the curves follow different courses. It might be pointed out here that in the original paper of Ansell and Dohmen[2] the phosphatidic acid is referred to as an 'unknown phospholipid' yielding glycerophosphate on mild alkaline hydrolysis, because of the doubt then existing about the presence of phosphatidic acid as an *in vivo* component of tissues. Meanwhile, Hokin and Hokin[14] have resolved this problem and have shown that phosphatidic acid is present in brain tissue, though their results suggest that its uptake of phosphorus-32 over a short interval *in vivo* is slower than we have found. Hübscher and Clark have also unequivocally obtained a phosphatidic acid from liver[15].

It is, however, admittedly difficult to make comparisons when animals of different ages are involved. For the rat *in vitro*[16] and for the rat and mouse *in vivo*[1,17] it has been shown that the capacity of the brain to incorporate orthophosphate into phospholipids declines rapidly from birth to early maturity; thereafter the rate decreases much less sharply. Recent experiments have shown that brain sphingomyelin incorporates radioactive phosphate much more rapidly in the myelinating rat brain than in the older brain when myelin is not being so actively formed[18]. It is apparent, in fact, as might be expected, that the rate of replacement of the phosphate moiety

of brain phospholipids varies with age and with the phospholipid under consideration.

[1] Dawson, R. M. C., and Richter, D., *Proc. Roy. Soc.*, B, **127**, 252 (1950).
[2] Ansell, G. B., and Dohmen, H., *J. Neurochem.*, **2**, 1 (1957).
[3] Davison, A. N., and Dobbing, J., *Biochem. J.*, **73**, 701 (1959).
[4] Marinetti, G. V., Erbland, J., Albrecht, M., and Stotz, E., *Biochim. Biophys. Acta*, **30**, 543 (1958).
[5] Ansell, G. B., and Dawson, R. M. C., *Biochem. J.*, **50**, 241 (1951).
[6] Ansell, G. B., and Spanner, S., *J. Neurochem.*, **4**, 325 (1959).
[7] Miani, N., and Bucciante, G., *Atti. Soc. Med. Chir. Padova*, **34**, 5 (1956–57).
[8] Dawson, R. M. C., *Biochim. Biophys. Acta*, **14**, 374 (1954).
[9] Ansell, G. B., and Dohmen, H., *J. Neurochem.*, **1**, 150 (1956).
[10] Dziewiatkowski, D., and Bodian, D., *J. Cell. Comp. Physiol.*, **35**, 141 (1950).
[11] Samuels, A. J., Boyarsky, L. L., Gerard, R. M., Libet, B., and Brust, M., *Amer. J. Physiol.*, **164**, 1 (1951).
[12] Changus, G. W., Chaikoff, I. L., and Ruben, N., *J. Biol. Chem.*, **126**, 493 (1938).
[13] Davison, A. N., and Dobbing, J., *Lancet*, 1158 (1958).
[14] Hokin, L. E., and Hokin, M. R., *J. Biol. Chem.*, **233**, 800 (1958).
[15] Hübscher, G., and Clark, B., *Biochem. J.*, **72**, 7*P* (1959).
[16] Fries, B. A., Schachner, H., and Chaikoff, I. L., *J. Biol. Chem.*, **144**, 59 (1942).
[17] Fries, B. A., Changus, G. W., and Chaikoff, I. L., *J. Biol. Chem.*, **132**, 23 (1940).
[18] Ansell, G. B., and Spanner, S., *Biochem. J.*, **73**, 3*P* (1959).

METABOLISM OF POLYPORES WITH PHOSPHORUS-32

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In these studies, our object is to trace the migration of phosphorus-32 from the host-plant to the fungus as well as its migration from the surface of the fruit-body of the fungus to its hymenial layer by direct application on the upper surface of the fruit-body. No such study has been carried out with higher fungi, so far as can be ascertained from published reports. Recently, Grossbard[1] discussed autoradiography in the metabolism of fungi, showing its application in the physiology of some fungi (mostly lower) and pointing out its future usefulness in mycology and plant pathology.

Phosphorus-32 has been one of the most widely used radioisotopes on account of the biological importance of the element, as it is well known that phosphorus enters into the constitution of protoplasm, of phospholipids, phosphoproteides, amino-acids, and neucleoproteides (cellular metabolism); moreover, phosphorus-32 can be easily produced, it has convenient half-life (14·3 days) and it emits energetic β-rays.

We first followed absorption of phosphorus-32 by *Fomes* (*Polyporus*) *lignosus*. *F. lignosus* is usually known as a common root disease-fungus. Its habitat, distribution, morphology and cultural characters have been fully described by one of us[2].

We adopted two concentrations of phosphorus-32 (solution of phosphorus-32 without carrier in neutral solution of sodium phosphate (*p*H 7) prepared by C.E.A. in sterilized vessels) for our study; 5 μc. and 50 μc. per litre; this activity is sufficiently low as not to disturb the metabolism of the fungi, and with this it is convenient to have reasonably accurate radioactivity measurement. Fresh carrot roots were surface-sterilized with calcium hypochlorite (200° chlorometric per kgm.) for 1½ hr., then washed thoroughly with four or five changes of sterilized water at intervals of 20 min. in order to eliminate the toxic matter completely. The outer surface (peripheral tissues) of such roots, which turned white and translucent due to the treatment, was rejected. They were cut aseptically into pieces of 3–4 cm. in length and put (leaf-side up and root-side down) inside sterilized Roux tubes, constricted ends of which contained Gautheret's[3] liquid medium supplemented with phosphorus-32 (sodium phosphate) of the above two doses; each Roux tube contained one piece of the carrot, and the lower end of each piece was kept dipped in the liquid medium for direct absorption of phosphorus-32. A large number of Roux tubes was thus prepared for tissue culture of carrot root, and after an interval of 15 days, when there was good absorption of phosphorus-32 by the carrot according to the Geiger–Müller count (in many cases there was a small growth of new tissue (callus) at the cut surface of carrot pieces), the Roux tubes containing the carrot pieces were exposed to a constant temperature of 60° C. for 20–30 min. After cooling, each carrot piece was inoculated with a very young hyphal culture of *F. lignosus*, the inoculum being approximately equal in all cases. The fungus (a weak parasite) does not grow easily on living mature carrot roots on account of the tissue-resistance, though it kills at once very young tissue cultures; but it has been found that, if the resistance is lowered by exposure to 60° C. for about half an hour, the fungus makes a vigorous growth (Fig. 1) in the course of two or three days. Now, after an interval of 8–10 days, the measurements of activity of the fungus and of carrot pieces were taken; the activity of the fungus growing on carrot with concentration of 50 μc. and 5 μc. per l. was distinct; detailed results are given in Table 1*a*. This is an example of two techniques together: the technique of tissue-culture of carrot and that of radioisotope of phosphorus-32.

We then studied absorption of phosphorus-32 from solid medium with 1 per cent agar in Petri plates in Gautheret's medium, to which two doses of 5 μc.

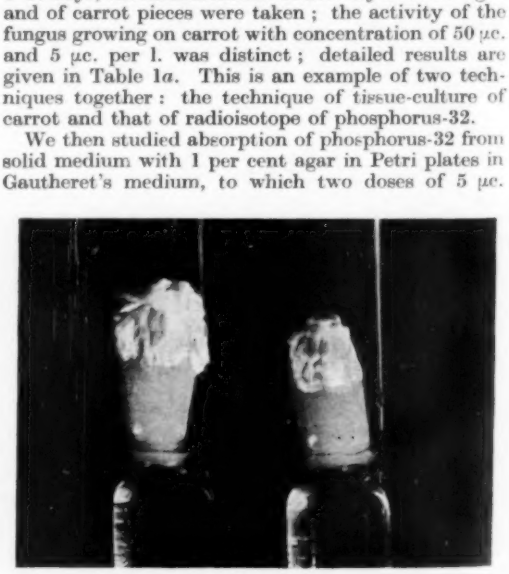

Fig. 1

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Table 1. RADIOACTIVITY OF SAMPLES

(*a*) Activity of fresh tissue.			
Carrot (5 μc./litre)		4·02	counts/min./mgm.
Fungus (*Fomes lignosus*)	(50 μc./litre)	270	,,
	(5 μc./litre)	389	,,
(*b*) Activity of fresh tissue.			
Agar medium (50 μc./litre)		4·8	,,
Fungus (*F. lignosus*)		69·5	,,
(*c*) Activity of spore-slides of *Ganoderma applanatus* var. *australis*.			
Sporophore *A* (mean of 22 slides)		9·6	counts/min./slide.
Sporophore *B* (mean of 15 slides)		29·2	,,
(*d*) Activity of sporophore-tissues of *G. applanatus* var. *australis*.			
Sporophore *B*	context area	24·2	counts/min./cm.²
	porous area	83·7	,,
Sporophore *C*	total area	102	,,

per l. and 50 μc. per l. of phosphorus-32 were added, it being solidified with 1 per cent agar; 10 c.c. of the medium was poured on 9 cm. plates, which were autoclaved, and circular sheets of sterilized 'Cellophane' paper and filter paper equal in diameter to that of the bottom half of a Petri dish were aseptically placed on the surface of the medium, one to each plate. Equal pieces of inocula from the growing edge of a very young plate culture of *F. lignosus* aseptically cut with a cork borer of 8 mm. diameter, were placed on the paper in the plates. After allowing the fungus to grow and absorb phosphorus-32 for 12–20 days, the absorption of the isotope by the fungus at different dates within the interval was measured from the specific activity of the samples according to Geiger–Müller count. The absorption was found significant and that by the bare filter paper was much less than the absorption by the fungal hyphæ; detailed results are given in Table 1*b*.

More interesting results were obtained by application of phosphorus-32 to an intact plant (a *Tilia* tree growing in the compound of the Château de Gif at the Centre National de la Recherche Scientifique) which bore nine small fruit bodies of *Ganoderma applanatus* var. *australis* on the body of the trunk within a length of about 4 ft. above ground-level (Fig. 2 marked with arrows). Scott Russell[4] holds the appearance of radioactivity in shoots of intact plants can provide unequivocal evidence of active transport. Tukey[5] has shown that radioactive minerals entered even through the bark of fruit trees (apple and peach) quickly, and that within 24 hr. of application, radioactivity was detected within the dormant branches, 18–24 in. above and below the points of application, even during winter with temperatures below freezing point.

On July 11, 1958, two square holes (2 cm.²) were drilled in the bark of the trunk of the above-mentioned living tree (*Tilia* sp.) at a height of about 4 ft. from its base, just above and below a sporophore (*A*) of *G. applanatus* var. *australis*, and two cotton pads (2 cm.²) soaked with 2 c.c. of phosphorus-32 (dose being 100 μc. per 2 c.c.), were inserted into the holes, one to each hole; now a number of slides smeared with glycerine were placed below the sporophore on a thick 'Cellophane' platform to catch the falling spores which were being shot out copiously and continuously day and night from the beginning of July, and the whole was very carefully covered with 'Cellophane' paper to keep out external draught or rain. Another cotton pad similarly soaked with phosphorus-32 of the same dose was placed on the top surface of another sporophore (*B*) on the same trunk, and slides (smeared with glycerine) were placed beneath the sporophore, the whole being similarly covered with 'Cellophane' paper. An adjacent sporophore (*C*) just 4 in. below the sporophore (*A*), to which no phosphorus-32 was applied in any way, was kept for observation with slides beneath the sporophore to catch the spore-fall. After an interval of 4 days, slides with copious spore-deposits from the three sporophores *A*, *B* and C, as well as fragments of three fruit bodies with porous areas, were taken out for measurement of radioactivity. In each case of the spore-slides and pieces of sporophore the activity was significant, and shot-out spores were distinctly radioactive, while spore-deposit from the same fungus taken two weeks before the application of phosphorus-32 to the trunk showed no radioactivity; detailed results are given in Table 1*c*. The porous areas were more radioactive than the context areas (Table 1*d*). The radioactivity of the sporophore *C*, to which there was no application of phosphorus-32, goes to show that there was active transport of phosphorus-32 in the body of the tree as remarked by Tukey.

On October 2, 1958, at 11 a.m., a second higher dose of 1 mc. per 2 c.c. of phosphorus-32 was injected into the top hole only of sporophore *A* (*G. applanatus* var. *australis* growing on the same *Tilia* tree at the Château de Gif), and a 2 per cent agar opened (cover taken out) plate and 10 aluminium cupules were aseptically placed underneath the sporophore at 3 p.m. Geiger–Müller counting after 20 hr. showed that spores were positively radioactive of the order of the background. Before this second injection, spore-fall of *G. applanatus* var. *australis* on slides smeared with glycerine was taken for 24 hr. during September 14–15, 1958; such spores, when tested, were found to be not radioactive. This occurred after an interval of two months and four days from the first injection. Spore-fall from the sporophore *A*, which was radioactive, occurred only once, on October 3; and it entirely stopped thereafter, that is, one month earlier than the normal time, whereas the spore-fall from the sporophore *B* (which was not radioactive) on the same tree continued up to November 6. Germination of spores which were not

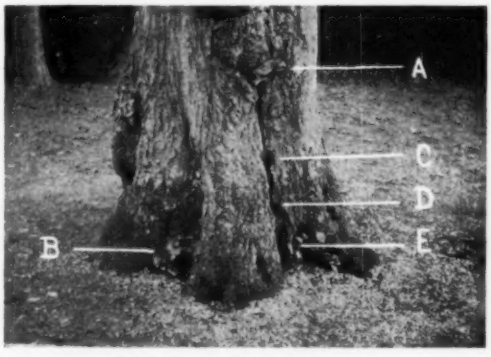

Fig. 2

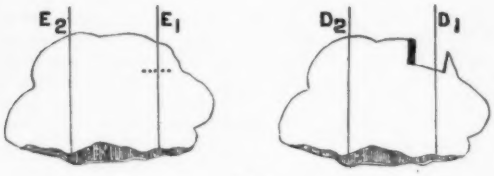

Fig. 3

Fig. 4. *D*1. 10 min. exposure

Fig. 5. *D*2. 10 min. exposure

Fig. 6. *E*2. 10 min. exposure

radioactive, in malt-agar tubes, was quite easy; they germinated in the course of 10 days, whereas germination of radioactive spores took place only once, after an interval of 20 days, and in one malt agar tube only out of ten such tubes; but cultures of these radioactive spores in malt agar plates showed no trace of radioactivity. Moreover, from examination of colour change of the hymenial surface of the sporophore *A* from a whitish to a brownish colour after October 3, it appears that it had then stopped throwing out spores (Bose[4]). It was further noted that subcultures of radioactive spores in malt agar plates in the dark at 24° C. (incubator) grew perceptibly more slowly than those of normal non-radioactive spores of *G. applanatus* var. *australis*; the diameter of 10 days growth of the mycelial mat in malt agar plates was only 6 cm. in the case of radioactive spore-hyphæ, whereas in the case of non-radioactive spore-hyphæ the diameter of growth under identical conditions was 8·5 cm. on the average. But detailed examination of the growth of the two mycelial mats under the microscope showed no difference whatsoever in characters of the hyphæ; thus the noted difference in rate of growth may be purely cytoplasmic, as indicated by Jinks[7]. Consequently, it may be held that radioactive spores of *G. applanatus* var. *australis* are susceptible to radiation.

Microscopical examination of the surface of the hymen of normal sporophores showed that, by November 1958, they were past the sporing stage, basidia being almost entirely replaced by tramal hyphæ—elongations which rapidly fill up the pores—and *Ganoderma* spores are no longer found in sections of sporophores.

On December 12, two sporophores (*D* and *E*) of *G. applanatus* var. *australis* growing on the same *Tilia* tree at Gif Château were treated with phosphorus-32, the dose being 1 mc./c.c. in each case. In the case of sporophore *D* the very tough upper surface was scratched to a depth of 1 cm., and a cotton pad soaked with 1 c.c. of phosphorus-32 was placed directly on the scratched surface, while in the case of sporophore *E*, the upper surface was injected with a tuberculin syringe, 1 cm. deep, 1 c.c. of phosphorus-32 being divided into four injections of equal quantity of the liquid. Both the sporophores, *D* and *E*, were very carefully covered with 'Cellophane' and brown paper for protection against the prevailing light showers of rain and occasional gusts of wind. After an interval of four and a half days, each of the two sporophores (*D* and *E*) was cut longitudinally at two spots—one corresponding to the treated areas (*D*1 and *E*1), and the other, distant areas (*D*2 and *E*2); so, they were marked respectively as *D*1 and *D*2 and *E*1 and *E*2 (Fig. 3).

These four pieces were then autoradiographed at Saclay, Service de Biologie du Centre d'Etudes nucleaires, following closely the method of Pellerin[8]. The injection method was not quite satisfactory on account of suspected leakage from the needle-end pressing against the very tough surface of the sporophore, but in the distant area, *E*2 (Fig. 6), there was a clear indication of accumulation of phosphorus-32 towards the hymenial surface. The specimen-pieces were highly radioactive according to Geiger–Müller count—*D*1 giving 49,000 counts per min. per gm., and *D*2 giving 45,000 counts/min./gm.

An exposure of 10 min. to X-ray film in the dark with liquid nitrogen at − 200° C. was sufficient to bring out distinct images (Figs. 4, 5 and 6). It can be seen that heavy accumulation of phosphorus-32 takes place towards the hymenial (porous) surface. There is thus clear evidence of ready transport of phosphorus-32 to the most active area, full of living cells. Control (a longitudinal section of a normal sporophore) under identical conditions showed a perfectly blank area.

One of us (S. R. B.) is grateful to the Centre National de la Recherche Scientifique, Paris, for a research grant for this work and to the authorities of the Institut du Radium (Laboratoire Curie) for facilities for this work, and we thank M. D. Basu working at Saclay (C.E.A.) for help with a portion of this work with tree injection and with part of the Geiger–Müller counting. For the mycological work one of us (S. R. B.) is indebted to Prof. R. Heim for facilities accorded in the Laboratoire de Cryptogamie, Muséum National d'Histoire Naturelle, Paris.

[1] Grossbard, E., *Nature*, **182**, 854 (1958).
[2] Bose, S. R., *Trans. Brit. Mycol. Soc.*, **40**, 456 (1957).
[3] Gautheret, R. J., "Manuel technique de Cultures des Tissus Vegetaux" (Masson et Cie, Paris, 1942).
[4] Scott Russell, R., "Progress in Nuclear Energy", Series VI, Biol. Sciences (Pergamon Press, **1**, 87, 1956).
[5] Tukey, H. B., and Wittwer, S. H., "Progress in Nuclear Energy", Series VI, Biol. Sciences (Pergamon Press, **1**, 106, 1956).
[6] Bose, S. R., *J. Ind. Bot. Soc.*, **23**, 155 (1944).
[7] Jinks, J. L., *C.R. Lab. Carlsberg., Ser. Physiol.*, **26**, 183 (1956).
[8] Pellerin, P., *C.R. Acad. Sci., Paris*, **214**, 1555 (1957).

LETTERS TO THE EDITORS

PHYSICS

Film Transfer in He II

DURING the course of experiments on film transfer in very narrow beakers and in vessels with very narrow apertures, phenomena have appeared which are difficult to reconcile with the existing interpretation of the nature of film transfer and of critical velocity[1].

It has been found, especially at temperatures in the neighbourhood of 1·3° K. where most of the measurements have been made, that the rate of transfer out of a narrow cylindrical beaker of, say, 1·5 mm. diameter is dependent on the history and condition of the liquid in the beaker and is no longer the characteristic rate of transfer per cm. width of surface at the temperature in question. Eselson and Laserev[2] observed a similar effect some years ago, but were unable then to offer an explanation. The present results are in agreement with their work and extend it in several ways. Recently, Snyder and Donnelly[3], describing film transfer from rotating vessels, also mention one or two instances of a similar effect.

For the experiments reported here a cylindrical beaker was used, 5 cm. long and of inner diameter 1·8 mm. and outer diameter 3·6 mm. The rim of the vessel was ground concave conically so as to give clearly defined outer and inner perimeters. A typical experiment consisted first of filling the beaker—either by plunging it beneath the bath or by allowing it to fill by film transfer—and then of observing a sequence of emptyings and fillings by film transfer alone, over a range of 4–15 mm. below the rim of the beaker. Table 1 shows the results of such an experiment. Sequence *A* was initiated by plunging the beaker and then withdrawing it; sequence *B* was initiated by emptying the beaker completely after *A* and then filling solely by the film; and finally, sequence *C* was initiated by a further plunge-fill of the beaker.

From this and similar experiments with other vessels the following general features were observed: (*a*) following a plunge-fill, the rate of emptying was higher than it was after a film-fill, the maximum observed difference between the two rates for the same beaker being nearly 70 per cent; (*b*) rates of filling were always lower than rates of emptying, the difference being to some extent dependent on the wall thickness; (*c*) for the same vessel, rates of emptying after successive plunge-fills differed from each other, sometimes by as much as 15 per cent; (*d*) a pause of 20 min. between a plunge-fill and subsequent emptying had no observable effect of reducing the fast rate of emptying.

Table 1

Sequence	Transfer-rate on emptying (cm.³ sec.⁻¹ × 10⁵)	Transfer-rate on filling (cm.³ sec.⁻¹ × 10⁵)
A (Plunge fill)		
Emptying	13·2	
Filling		6·87
Emptying	9·02	
Filling		6·82
Emptying	9·17	
B (Film fill)		
Filling		6·90
Emptying	8·57	
Filling		6·70
Emptying	9·47	
C (Plunge fill)		
Emptying	14·2	
Filling		6·42

It was inferred that the fast rate of emptying by the film following a plunge-fill drained off the excess of something which had been introduced by the plunge, and that this excess might be turbulence. This inference was tested in a separate experiment in which a small solenoid-activated iron wire stirrer was put into a beaker. It was observed that continuous stirring during emptying increased the rate of emptying by 10 per cent over the unstirred rate, while continuous stirring during filling reduced the normal filling rate by about 10 per cent. Although the reduction in filling rate was remarkable, the increase in emptying rate caused by stirring the beaker was not as great as the increase obtained by a plunge-fill, which might indicate that stirring is not an efficient way of making turbulence in so nearly an ideal fluid.

It has been assumed that there exists a 'critical' rate of film transfer, although its precise value has been a matter of some disagreement. Hence, knowing the thickness of the film one can determine a 'critical' velocity. From earlier experiments it has been inferred that when the velocity is less than 'critical' the liquid in the film moves without friction, but that at or a very little above the 'critical' velocity the friction becomes great and the energy is dissipated in the creation of excitations.

It is difficult to reconcile the notion of 'critical' velocity with the experiments that are reported here, and we may therefore be forced to abandon it. If so, it could be replaced by one in which the movement of the film is intimately associated with turbulence. Suppose, for example, that the film moves, as it were, on line vortex roller bearings, and that if no such vortices are present in the film then no motion of the film occurs. If this is so, then one might expect that the more vorticity there is available at the source of the film the more liquid will be transferred by the film, whereas if vorticity is scarce at the source then the rate of transfer will be lowered. Excess of turbulence at the downstream end of the film also appears to slow the transfer, which might indicate either that turbulence is flowing both ways in any film so that the transfer is a net transfer, or that the specific quantity of turbulence in a vessel has an appreciable chemical potential.

The picture, although it is obviously very crude, does not appear to be in conflict with earlier experiments on film transfer, and it may also have relevance for the concept of critical velocities in the bulk liquid. A so-called 'critical' velocity might cease to be the upper limit of velocity before the onset of friction, and become a velocity which depends on the energy required to produce the amount of vorticity involved in the flow.

This kind of vorticity or turbulence is very long-lived; in the example quoted earlier it appeared to

remain more or less intact for at least 20 min. in a volume of about 0·1 cm.³. The conversion of such vorticity into rotons, which was envisaged by Feynman[4], seems therefore to be a very slow process indeed.

In the particular case of film transfer one is involved in discovering the particular properties of line vortices running along close to a wall, and also whether in the film which covers the surface of a tube the vortices form closed rings or a continuous helix with one end fixed in the bath and the other in the beaker.

These results, together with others made with vessels with very narrow necks or with matt surfaces, will be more fully discussed in a later paper.

In conclusion, I wish to record my thanks to Dr. C. G. Kuper for much fruitful collaboration, and also to Dr. D. V. Osborne and Dr. D. Bohm for stimulating discussion.

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[1] See, for example, Atkins, K. R., "Liquid Helium" (Cambridge University Press, 1959).
[2] Eselson, B. N., and Laserev, B. G., *J. Exp. Theor. Phys.* (U.S.S.R.), **23**, 552 (1952).
[3] Snyder, H. A., and Donnelly, R. J., *Phys. Fluids*, **2**, 408 (1959).
[4] Feynman, R. P., *Prog. Low Temp. Phys.*, **1**, 17 (1955).

Mechanism of Superfluid Flow

Theories of superfluidity usually assume the possibility of pure potential flow, provided that the flow velocity is less than some critical value (at which the creation of excitations first becomes possible)[1,2]. If we consider the flow of helium (at $T = 0°$ K.), for example through a tube or along a Rollin film, it is implied that the boundary layer is slipping over the solid wall. However, the first few layers of helium atoms would not be expected to take part in this motion, as they are firmly bound by the van der Waals' attractions of the wall. The picture therefore has to be revised slightly to one in which there is a velocity discontinuity (that is, a vortex sheet) close to, but not coincident with, the solid wall. But a continuous vortex sheet implies a rather drastic change in the character of the wave function, over a distance of the order of one interatomic distance a, and hence implies a large energy per unit area, of the order of $\hbar^2/ma^4$ (where m is the mass of an atom).

A more realistic model for helium, flowing at $T = 0°$ K., would seem to be one in which the vortex sheet is replaced by some sort of array of Onsager–Feynman quantized vortex lines. For example, a Kármán row of unit quantized vortices[3] will be able to reproduce the macroscopic features of the 'vortex sheet' model for flow parallel to a plane wall; the vortices will themselves take part in the motion. The liquid in contact with the wall should have zero average velocity and reasonably small actual instantaneous velocity.

Let us consider a row of vortex lines of strength $\varkappa = 2\pi\hbar/m$, perpendicular to the plane of the paper, cutting it at the points (na, b), and let the solid surface be the plane $y = 0$ (Fig. 1). Then, if the vortices are at rest, the x and y components of the velocity are given by the real and imaginary parts respectively of[3]:

$$-i\pi\varkappa a^{-1} \cot\{\pi a^{-1}(x + i(y - b))\} \qquad (1)$$

If $a \ll b$, this pattern approximates to a vortex sheet, and the oscillatory part of the velocity is very small

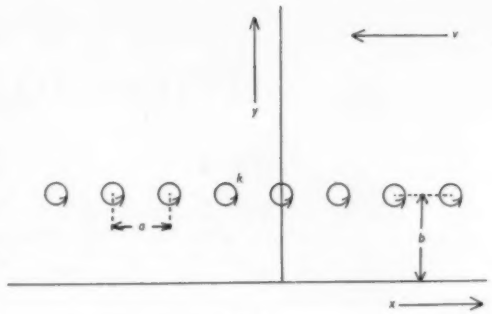

Fig. 1

except very near the vortex row; indeed, even if a is fairly comparable to b, provided only $\exp(-b/a) \ll 1$ holds, the periodic fluctuations of velocity at the wall remain small compared to the zero-point velocities of helium atoms. The mean velocity on a plane parallel to the wall is:

$$-\pi\varkappa a^{-1} \tanh\{\pi(y - b)/a\} \qquad (2)$$

and is in the x-direction. The boundary layer can be brought to rest, on the average, by superimposing a velocity:

$$-\pi\varkappa a^{-1} \tanh(\pi b/a) \qquad (3)$$

on the fluid (and in particular on the vortex lines). The magnitude of the macroscopic velocity of flow is then:

$$V = 2\pi\varkappa a = 4\pi^2\hbar/ma \qquad (4)$$

provided $b \gg a$, and the depth of liquid $\gg b$.

In a Rollin film of thickness t, if $a \gg t$, flow by this mechanism cannot proceed—there are not enough vortices to serve as 'bearings' (Allen, J. F., previous communication). Hence a film cannot flow steadily unless $a \lesssim t$, when:

$$V \gtrsim 4\pi^2\hbar/mt \qquad (5)$$

Thus, instead of a 'critical' velocity, $\hbar/mt$ plays the part of a minimum flow velocity[4]. Any higher velocity is possible, but in general energy will have to be supplied to replace the vortex lines carried away by the flow. For the occurrence of lower velocities, a more complicated configuration of vortices will be needed, which will probably lead to increased dissipation. Some instability of flow might also be expected near the velocity (5).

Allen has suggested that his experimental results should be interpreted as showing that a Rollin film flows by 'rolling' on vortices. The microscopic arguments advanced here lead to just this picture of the flow. Older experiments, which appear to support the idea of a critical velocity, require re-interpretation. The present suggestion is that there is a velocity of minimum energy dissipation—the velocity which is normally observed in steady flow. It should be noted that a persistent current around a torus remains possible, since vortex rings can just roll round the torus.

The actual geometrical array of vortices is likely to be more complicated than I have envisaged here. The energy of interaction between two vortex lines is quite comparable to Feynman's[3] self-energy of one line, so that quantitative calculations become difficult. The mutual repulsions between like parallel vortices will probably tend to give a fairly uniform lattice of lines, rather than just a row.

I am greatly indebted to Prof. J. F. Allen for many discussions during the progress of his experiments and wish also to thank Dr. D. Bohm and Dr. O. Penrose for stimulating discussions.

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[1] Landau, L. D., *J. Phys.* (U.S.S.R.), **5**, 71 (1941); **11**, 91 (1947). Feynman, R. P., *Phys. Rev.*, **94**, 262 (1954).
[2] Feynman, R. P., "Low Temperature Physics", **1**, 17 (North Holland Pub. Co., Amsterdam, 1955). Onsager, L., *Nuovo Cim.* (ix), **6**, Supp., 249 (1949).
[3] See, for example, Milne-Thomson, L. M., "Theoretical Hydrodynamics", 340 (Macmillan, London, 1948).
[4] Contrast with the conventional interpretations; see, for example, Atkins, K. R., "Liquid Helium", 90 (Cambridge University Press, 1959).

Measurements of the Frequency of an Ammonia Maser in England and Australia

THIS communication describes preliminary measurements of the oscillation frequency of the Signals Research and Development Establishment Maser No. 5 at the National Physical Laboratory, Teddington (by the Standards Division), and afterwards at the Research Laboratories of the Australian Post Office in Melbourne, Australia. Maser No. 5 is a rack-mounted experimental maser with continuous pumping and employs unpurified commercial anhydrous ammonia. An eight-pole state separator is used together with a silver-plated brass microwave cavity operating in the TM_{010} mode. The cavity is thermally tuned to the (3,3) transition. A more detailed description of this maser may be found elsewhere[1].

The maser was operated at the National Physical Laboratory under the following conditions: Ammonia gun pressure 1·0 mm. mercury (corresponding to a total flow into the system of 3×10^{17} mole./s.) and 18 kV. d.c. between adjacent poles of the state separator. The resonant frequency of the cavity was adjusted thermally to a value such that the maser oscillation frequency changed less than 2 parts in 10^{10} when the gun pressure was changed from 0·5–1·0 mm. mercury. This was one of several possible methods of establishing a resettable oscillation frequency. Other methods give different oscillation frequencies[1,2], none of which necessarily corresponds to the emission line frequency. The oscillation frequency was measured in terms of a local 5 Mc./s. quartz standard which in turn was calibrated against the cæsium resonator[3]. Results are shown in Table 1 (sets 1 and 2), the absolute accuracy of the National Physical Laboratory measurements being ± 2 parts in 10^{10}. After completion of set 1 the state separator and ammonia gun assembly were removed and the wave-guide connexion to the cavity uncoupled. This was an attempt to simulate the dismantling required prior to shipment of the maser by sea to Melbourne. After re-assembly the maser frequency was re-measured with the results shown as set 2. The downward shift in frequency of 1·3 parts in 10^9 is significant but time did not permit investigation of the reason.

The maser was then dismantled, shipped to Melbourne and installed at the Australian Post Office Research Laboratories. Certain improvements were made to the thermostat controlling the microwave cavity temperature and the cavity frequency again adjusted so that the maser oscillation frequency was independent of gun pressure changes between 0·5 and 1·0 mm. mercury. As before, the gun pressure was maintained at 1·0 mm. mercury and the separator voltage at 18 kV. d.c., while measurements of oscillation frequency were made in terms of a local 100 kc./s. quartz standard. The results are shown as sets 3–8, the spread of measurements within a set (col. 6) being slightly inferior to that obtained at the National Physical Laboratory. The frequency of the local quartz standard was measured by comparing it with the G.B.R. transmission from Rugby using a beat frequency method. Because of the poor signal-to-noise ratio of the received signal it was necessary to average the beats between G.B.R. and the local standard over a period of 22 hr. each day to obtain an accuracy of ± 4 parts in 10^{10}. However, an additional but unknown error of perhaps several parts in 10^9 must also be added due to propagation variations over the path Rugby–Melbourne. After applying the National Physical Laboratory corrections to the oscillator controlling G.B.R., the mean maser frequencies shown in sets 3–8 are obtained. The grand average of all the Melbourne results is 2·2 parts in 10^9 higher than the average of the National Physical Laboratory results. As stated above, this frequency difference is comparable to the propagation error which must be attached to the Melbourne mean frequencies given in Table 1.

Some evidence has been obtained to show that the frequency of oscillation is dependent on the ambient temperature to the extent of −1 part in 10^{10}/deg. C. rise. This is almost certainly due to inadequacy of the thermostat controlling the cavity temperature. The effect can be seen in sets 6, 7 and 8. The ambient temperature was rising throughout November 11, 1959, and was higher during measurements on November 12, 1959. Further work is being carried out both at the Signals Research and Development Establishment and the Australian Post Office Research Laboratories, Melbourne, towards discovering and controlling the residual factors governing the maser oscillation frequency. For comparison pur-

Table 1. SUMMARY OF FREQUENCY MEASUREMENTS ON SIGNALS RESEARCH AND DEVELOPMENT ESTABLISHMENT MASER NO. 5
Assumed cæsium frequency = 9 162 631 770 c./s.

Measurement set No.	Maser location	Date	Mean maser frequency (c./s.)	No. of readings	Standard deviation (parts in 10^{10})	Deviation of mean frequency from set 2 (parts in 10^9)
1	Teddington	14. 5.59	23 870 129 145	46	2·6	+ 1·3
2	,,	20. 5.59	,, ,, ,, 115	82	1·9	0
3	Melbourne	6.11.59	,, ,, ,, 197	5	3·2	+ 3·4
4	,,	9.11.59	,, ,, ,, 178	40	3·0	+ 2·6
5	,,	10.11.59	,, ,, ,, 196	41	2·8	+ 3·4
6	,,	11.11.59	,, ,, ,, 184	41	3·6	+ 2·9
7	,,	11.11.59	,, ,, ,, 167	47	4·1	+ 2·2
8	,,	12.11.59	,, ,, ,, 149	20	6·2	+ 1·4
—	Neuchâtel	—	,, ,, 128 870	—	—	−10·3

poses we have appended to Table 1 the published frequency[4] for an ammonia maser employed at Laboratoire Suisse de Recherches Horlogères, Neuchâtel, using another re-setting criterion. The frequency of this maser is considerably lower than that of Maser No. 5 and illustrates the fact that it is possible to operate ammonia masers with considerably different but yet highly stable oscillation frequencies.

We wish to thank the Director of the National Physical Laboratory for making available the results of the maser/cæsium comparisons obtained at Teddington. This communication is published with the permission of the Chief Scientist, Ministry of Aviation, and the Engineer-in-Chief, Post Master General's Department, Commonwealth of Australia.

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[1] Mitchell, A. M. J., Roots, K. G., and Phillips, G., "Electronic Technology" (to be published).
[2] Barnes, F. S., *Proc. Inst. Rad. Eng.*, **47**, 2085 (1959).
[3] Essen, L., and Parry, J. V. L., *Trans. Roy. Soc.*, **250**, 45 (1957).
[4] Observatoire de Neuchâtel, Suisse, Circulaire No. 2 (May 1959).

Monocular 'Rivalry' between Stabilized and Unstabilized Retinal Images

It is well known that images stabilized against retinal tremor tend to fade from vision[1,2], and it has been suggested that this indicates some form of fatigue or adaptation in the retinal receptors, which is normally relieved by small irregular displacements of the image. When using a simple stabilizing device[3] for the study of complementary patterns[4], I have observed a related effect which seems to require further, if not different, explanation.

The apparatus[3] comprises a close-fitting contact lens with an optical power of 60 dioptres, supporting a light 'Dural' tube on which objects under examination are mounted at the focal distance of about 1½ cm. The lens incorporates an artificial pupil 4 mm. in diameter. When the image of a sharply black-and-white object such as my 'ray' figure[4] is thus stabilized on the retina, fading is much less pronounced and permanent than with more delicate and irregular gradations of stimulus intensity[2].

The observation in question is as follows. When a thin object, such as a wire the diameter of which subtends perhaps 10′, is viewed in silhouette, it often remains visible for some time. If, however, an object giving an unstabilized image, such as the finger-tip, is now brought up just behind the wire to the focal zone of the stabilizing lens, a striking change takes place. The stabilized wire disappears as a whole, and the finger-tip appears completely visible, as if nothing were between it and the eye. The impression is similar to that given by binocular rivalry between disparate images in left and right eyes. When the finger is removed, the wire reappears.

With stabilized objects of increasing width, it is necessary (as might be expected) to scan the unstabilized background with an increasing amplitude if the objects are to disappear from view, and a limit is reached (dependent partly on the structure of the background) beyond which total disappearance does not occur.

It may well be suspected that the reluctance of the wire (and indeed of the 'ray' figure) to disappear permanently when stabilized is due in part to imperfect stabilization. This I leave an open question, though it may be remarked that with apparatus which presents the stabilized image within an unstabilized frame[1,2] it would now seem unsafe to use extinction of the image as a criterion of stability. What seems significant is that one can cause the disappearance—sudden and complete—of a thin stabilized object merely by presenting it against an unstabilized background. Together with the inhibition of extinction by regularity and boldness in the stabilized pattern this raises doubts whether changes in retinal receptors alone are responsible for the normal fading. On a retinal theory one might have expected that the addition of an unstabilized background would revive activity in neighbouring receptors adapted to a stable image by disturbing them, as flickering light is found to do[5].

It is difficult to see how it could predict the opposite effect actually observed, particularly the simultaneous and sudden disappearance of all parts of the wire. For this the idea of fatigue or adaptation among primitive gestalt-forming mechanisms would seem worthy of exploration.

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[1] Ditchburn, R. W., and Ginsborg, B. L., *Nature*, **170**, 36 (1952).
[2] Riggs, L. A., Ratcliff, F., Cornsweet, J. C., and Cornsweet, T. N., *J. Opt. Soc. Amer.*, **43**, 495 (1953).
[3] MacKay, D. M., *Nature*, **180**, 1145 (1957).
[4] MacKay, D. M., *Nature*, **180**, 849 (1957).
[5] Ditchburn, R. W., and Fender, D. H., *Opt. Act.*, **3**, 128 (1955).

GEOPHYSICS and GEOLOGY

Palæomagnetism in Antarctica

We have recently measured the direction of magnetization of two dolerite sheets and ten basic dykes in the Wright Valley (lat. 77·5° S., long. 161·5° E.) of South Victoria Land. The dykes intrude the basement granite in the area but do not cut the overlying Beacon Sandstone. The dolerite sheets are intruded into the basement and in some places cut the Beacon Sandstone. They post-date the dykes and the sandstone. The directions and relevant statistics are given in Table 1.

There is good evidence that the directions of magnetization are stable. The divergences of the mean directions of both sheets and dykes from the present Earth's field and the dipole field exceed the errors ($P = 0{\cdot}05$) by a factor of 3 or more. The coercivity of saturation isothermal remanence is high, varying from 600 to 1,200 œrsteds. Treatment in alternating magnetic fields of up to 370 œrsteds (peak) produced only small changes in the directions of magnetization. In some cases the directions remained little changed in alternating fields up to 750 œrsteds. These changes vary between 2° and 5° and are of the same order as the experimental error.

Other pole results from Antarctica have recently been reported by Nagata and Shimizu[1] from the Pre-Cambrian gneiss of Ongul Island (namely, lat. 3° N.,

Table 1. Mean Directions of Magnetization and Pole Positions

Geological unit	N	n	s	Mean direction of magnetization				Pole position				Divergences	
				D	I	R	α	Latitude	Longitude	$\delta\psi$	$\delta\chi$	ΔF	ΔP
Dolerite sills	8	11	20	262	−70	7·889	7	51° S.	132° W.	10	12	23	21
Basic dykes in grey granite	10	14	31	237	−56	9·832	7	29° S.	149° W.	7	10	32	38

N denotes the number of sites, n is the number of samples collected and s is the number of specimens cut from these samples. D and I are the declination and inclination of the resultant direction of magnetization computed by giving each site unit weight. R is the length of the resultant, α is the error of the mean at $P = 0{\cdot}05$ (Fisher, ref. 4), and ΔF and ΔP are the divergences of the mean direction from the present Earth's field (155°, −83°) and from the dipole field (0°, −84°) respectively. The pole positions in the southern hemisphere are specified by latitude and longitude and the errors $\delta\psi$ and $\delta\chi$ are in the direction of and at right-angles to the co-latitude, respectively.

long. 107° W.), by Turnbull[2] from the Cainozoic volcanics of Cape Hallett (lat. 81° S., long. 94° E.), dolerite sills of the Ferrar Glacier (lat. 58° S., long. 142° W.) and from the Beacon Sandstone (lat. 53° S., long. 151° W.), and by Blundell and Stephenson[3] from some dolerites from Theron Mountains and Whichaway Nunataks (lat. 54° S., long. 136° W.). These poles, together with those calculated from our results, are plotted in Fig. 1. The results from the dolerites are in good agreement. Turnbull is of the opinion that the Beacon Sandstone he sampled may have been reheated by the dolerite, which thus accounts for the agreement of the result from the Sandstone with the dolerites.

The precise geological age of these rock formations is not known. The basement dykes could be Palæozoic, the Beacon Sandstones are probably for the most part Upper Palæozoic or Early Mesozoic, and the dolerites are very likely to be Mesozoic in age. However, except for 4*C*, about the age of which little can be said, the relative age sequence is almost certainly that given in Fig. 1. The following general points may therefore be made.

(1) The divergence of the palæomagnetic pole from the present geographical pole increases with geological age. In geological recent times it is in the vicinity of the present pole and in the Pre-Cambrian it is near the present-day equator (see ref. 1 for discussion of the latter point).

(2) The poles have characteristic longitudes between 100° and 150° W. In these two features the results are similar to the pole paths obtained from Europe, North America, India and Australia.

(3) However, the longitudes differ very greatly (in some cases by 100° or more) from the longitudes of the poles obtained from these four regions. This result emphasizes for yet another continent the divergence that occurs between results from different continents for periods prior to the Tertiary.

Fig. 1. Pole positions from Antarctica. The pole positions are numbered as follows: 1, Ongul Island gneiss; 2, basement dykes; 3, Beacon Sandstone; 4*A*, dolerite sills, Ferrar Glacier; 4*B*, dolerite sheets of Wright Valley; 4*C*, Theron and Whichaway dolerites; 5, Hallett Cove volcanics. The projection is equatorial stereographic

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[1] Nagata, T., and Shimizu, Y., *Nature*, **184**, 1472 (1959).
[2] Turnbull, G., *Arctic*, **12**, 151 (1959).
[3] Blundell, D. J., and Stephenson, P. J., *Nature*, **184**, 1860 (1959).
[4] Fisher, R. A., *Proc. Roy. Soc.*, A, **217**, 295 (1953).

Infra-Red Determination of the Kaolin Group Minerals

By infra-red absorption analysis, it is possible to make a semi-quantitative estimation of the kaolin minerals. In contrast to X-ray diffraction, the infra-red spectrum of halloysite is as clearly defined as those of kaolinite and dickite, and the content of these three minerals in mixtures can be determined by a ratio between the depths of their hydroxyl absorptions. The peaks at 2·70μ (*A*) and 2·75μ (*B*) are common to all three minerals, and a peak depth ratio *A*/*B* of 1·2–1·5 is characteristic of kaolinite, with a ratio *A*/*B* of 0·8–0·9 characterizing halloysite. Values of 0·6–0·8 are typical of dickite. If the depth of the band due to physically adsorbed water at 2·9μ (*C*) is plotted as the abscissa with *A*/*B* as the ordinate, the three minerals fall into separate areas.

Examination of the spectra due to Si–O and Al–Si–O between 8·9 and 10·1μ show a diagnostic pair of doublets. The doublet at 10·7μ (*D*) and 11·0μ (*E*) is typical of the Al–O–OH bonding of kaolinite and dickite, although only the single peak at 11·0μ appears for halloysite. An inspection of the *A*/*B* ratio and the presence, or absence, of absorption (*D*) enables a unique distinction to be made between the three minerals. The *A*/*B* ratio remains constant during dehydration over several hours up to 450° C.

The absorption at 2·70μ (*A*) is found only in the kaolin group and in such simple structures as brucite. It is due to oxygen–hydrogen stretching frequencies associated with the hydroxyl ions, the oxygen and hydrogen atoms being in two symmetrical layers in

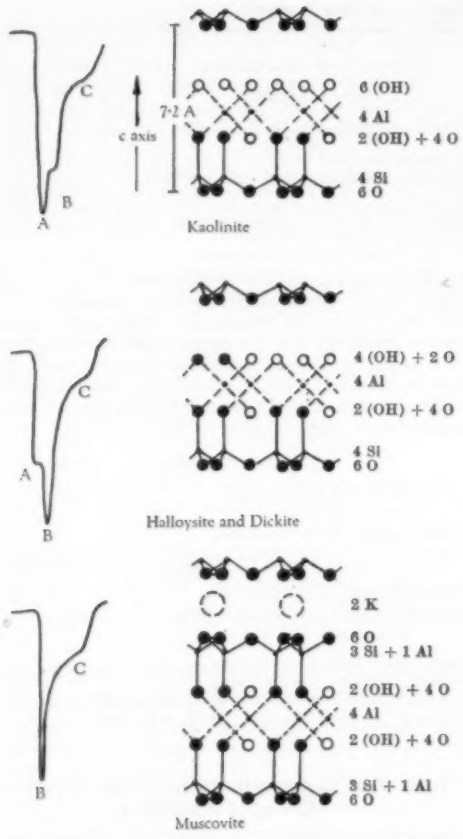

Fig. 1. Suggested oxygen–hydroxyl configuration for halloysite and dickite as compared with kaolinite and muscovite. Hydroxyl portions of their infra-red curves appear to the left of each structure

the brucite crystal. The 2·75μ (B) absorption is common to all micas and three-layer minerals, which differ from kaolinite in that their upper layer of oxygen–hydroxyls is only one-third hydroxyl, two-thirds oxygen.

A modification of the general kaolinite structure is proposed to explain the similarity in A/B ratio in halloysite and dickite as contrasted to that of kaolinite. The frequency of hydroxyls in the upper oxygen–hydroxyl level in the kaolin structure must vary between 3/3 (as in kaolinite) and 1/3 (as in the micas, when both upper and lower levels of the octahedral layer become symmetrical) (Fig. 1). Micas thus have a single (B) hydroxyl absorption. Kaolinite itself shows a full upper level of (A) type and full lower level of (B) type. Halloysite and dickite must lie at an intermediate value where 2/3 of the upper level are hydroxyls. This would decrease the strength of the (A) type absorption and increase the strength of the (B) type absorption, lowering the A/B ratio.

Aluminium–silicon substitution (Y number) in the basal, tetrahedrally co-ordinated levels of kaolinite, as proposed by Bates[1], should be very easy to determine in the same manner as used in the chlorite and other mica studies[2].

A more complete study on this subject will be presented at the forthcoming Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy in March 1960.

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[1] Bates, T., and Comer, J. J., Clays and Clay Minerals, Proc. Sixth Nat. Clay Conf., Nat. Acad. Sci.–Nat. Res. Council (1958).
[2] Tuddenham, W. M., and Lyon, R. J. P., *Anal. Chem.*, **31**, 377 (1959).

Sea-Level Fluctuations during the Past Four Thousand Years

Study of constructional details of a chenier plain along the coast of the Firth of Thames, New Zealand, together with radiocarbon dating of seven shell samples, has enabled the production of Fig. 1. Criteria used in interpreting the past sea-levels were (*a*) storm ridges, (*b*) high-spring-tide wash benches and (*c*) tidal stream flats. Often all three criteria could be used to determine one sea-level. Periods of sea-level highs correlate well with periods of transgression recorded along the European coast[1–3].

Tectonic movement, compaction and other causes could have led to misleading results; but their effects are considered small. Thus neglecting the minor fluctuations shown in Fig. 1, sea-level fell 7 ft. from 2000 B.C. to about the beginning of the Christian era and has remained relatively stable since. The present rise of sea-level is locally 8–9 in. per century and may well be only another minor fluctuation in an otherwise stable sea.

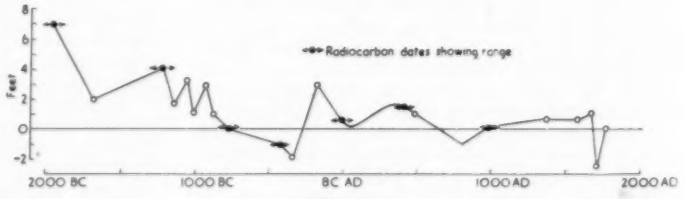

Fig. 1

Details will be published shortly in the *New Zealand Journal of Geology and Geophysics*.

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[1] Bakker, J. P., *Geologie en Mijnbouw.*, **16**, 232 (1954).
[2] Bennema, J., *Geologie en Mijnbouw.*, **16**, 254 (1954).
[3] Tavernier, R., and Moormann, F., *Geologie en Mijnbouw.*, **16**, 201 (1954).

Recognition of Folds and Faults on Rock Surfaces beneath the Sea

INFORMATION about the folding and faulting of the rock layers beneath the sea can be obtained by geophysical techniques, used in conjunction with sampling; but it is not easy to give a precise position of the geological boundaries on the floor, and only large faults can be inferred. The required detail can be obtained, however, by means of a geologists' asdic, where there are rock floors which consist of alternations of hard and soft beds and the former, being resistant to erosion, stand proud as ridges. In the English Channel, the Purbeck, Portland, Kimmeridge, Corallian beds and the ancient rocks occurring as shoals to east and west of Eddystone give good results, while Tertiary, Chalk and New Red Sandstone beds are too homogeneous to be suitable.

The method makes use of echo-sounding equipment, but instead of pointing the beam vertically beneath a ship it extends laterally and is tilted down and stabilized at about five degrees below the horizontal, so that echoes can be obtained from any ridges within a wide belt of floor parallel to the ship's course. The value of the method has already been described[1] and the equipment has been used on R.R.S. *Discovery II* to make reconnaisance surveys of sea-floors around southern Britain, with recent publication of some studies of sedimentary structures[2,3]. During these surveys rock outcrops of the type shown in Fig. 1 were found some miles south of the Dorset coast. The ship moved along a straight course (top of figure) covering two miles between the time marks, and examined a belt of sea-floor 800 yards wide, giving an exaggeration of scale of about four times for the range abeam. On this continuous plan view can be seen the dark bands of rock outcrops turning round in a gentle fold between the bottom right and left corners of the figure. In the upper part of the record a secondary beam which reached the sea floor beneath the ship gave a profile, in which an outcrop (black) terminates as a low cliff sloping away from the ship, at more than five degrees below the horizontal. The cliff marks the edge of a bed while the dip slope must be in the opposite direction and approximately at right angles to the line of outcrop of the bed. The continuity of any bed is interrupted by a parallel series of small faults, marked on the edge of the record with '*F–F*'. The pattern becomes increasingly blurred and vanishes as outcrops are progressively masked by loose sand at the right side of Fig. 1.

A complete acoustic and sampling survey of this ground, off south-eastern Dorset, has since been made and an account, which is in preparation, will be published elsewhere with Dr. D. T. Donovan as co-author.

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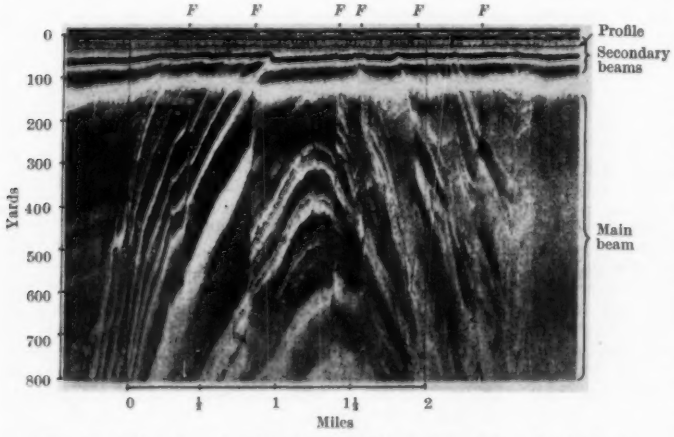

Fig. 1. A plan-view of rock outcrops (black) on the sea floor showing part of a gentle fold and small fractures, '*F–F*'

[1] Chesterman, W. D., Clynick, P. R., and Stride, A. H., *Acustica*, **8**, 286 (1958).
[2] Stride, A. H., *J. Mar. Biol. Assoc. U.K.*, **38**, 313 (1959).
[3] Stride, A. H., *Dock and Harb. Authority*, **40**, 145 (1959).

Dissolution of Silicate Rocks

IN work on the determination of trace elements in silicate rocks[1], an attempt has been made to solve the inherent difficulties of achieving a rapid and efficient dissolution of large samples (up to 200 gm.) of silicate rocks and at the same time ensuring that the minimum quantities of reagents are used.

The classical treatment of rocks involves dissolving the sample in a sulphuric/hydrofluoric acid mixture or a perchloric/hydrofluoric acid medium, the sample being contained in a platinum dish. There are several disadvantages inherent in the use of platinum ware for these operations, in particular the extremely high price of platinum apparatus which is slowly destroyed by fuming with sulphuric acid. A further serious objection is the fact that no determinations of any of the platinoids can obviously be carried out in such a container.

Since I have been concerned mainly with the determination of very rare elements in silicate rocks, appreciably large samples have had to be used (on the average about 20 gm.). The dissolution of much smaller quantities of rock (say 1 gm.) presents few difficulties[2] but clearly a special technique had to be employed for these larger samples.

The dissolution technique presented here is the result of several years experience and depends on the use of 'Teflon' (polytetrafluorethylene) containers. This plastic possesses several very significant properties including an almost complete resistance to chemical attack by most reagents except molten alkali metals. It also possesses a marked freedom from warping or softening at temperatures up to 300° C. This material is expensive (a 150-ml. beaker costs about £6) but will certainly outlast platinum and is only a fraction of the cost of this metal.

It is hoped that a description of the method will be of assistance to other workers, particularly in the geochemical sphere, and will help them to reduce the tedium of the dissolution stage of chemical analyses on silicate rocks.

The technique described step-wise below is based on a sample weight of 40 gm. and can be carried out in a 150-ml. 'Teflon' beaker. If greater or lesser quantities are required, *pro rata* volumes of reagents must be used. (*a*) Weigh 40 gm. of finely powdered sample (100 mesh or greater) into a 150-ml. 'Teflon' beaker. (*b*) Add 10 ml. of aqua regia and stir into a paste with a 'Teflon' rod. (*c*) Very cautiously add 50 ml. of 'AnalaR' hydrofluoric acid in small amounts of about 3 ml. each. This is a hazardous operation and care must be taken to avoid frothing. (*d*) Add a further 40 ml. of aqua regia and take to dryness in a water-bath. Because of the low heat transmittancy of 'Teflon', the beaker should be suspended actually inside the water-bath with the top of the beaker protruding about ¼ in. above the top of the bath. (*e*) When the sample is dry (usually about 24 hr.), add 50 ml. of hydrochloric acid and transfer contents to a 250-ml. borosilicate glass beaker. Add 150 ml. of water and boil for about 5 min., allow to settle for ½ hr. and pour off the supernatant solution. (*f*) Transfer the residue back to the 'Teflon' beaker and again add 50 ml. of hydrofluoric acid and 50 ml. of aqua regia. Caution in adding reagents is not necessary in this step. (*g*) Again take to dryness in the water-bath and combine the residue with the original decanted liquid. (*h*) Add 50 ml. of sulphuric acid to the solution and fume to dryness to remove excess fluoride ions. This step is carried out in a borosilicate glass beaker and slight etching of the glass may occur due to liberation of fluoride ions. This degree of etching is slight and may be ignored unless a determination of alkali metals is being carried out, in which case careful blanks are necessary. (*i*) Add about 200 ml. of 2 *N* hydrochloric acid to the residue and 1 ml. of bromine. Boil for 5 min. and add sufficient hydrochloric acid (2 *N*) to adjust the total volume to about 1,250 ml.

In most cases the above treatment will result in a clear solution. Some rock types, however, will not dissolve completely with this treatment and an appreciable sediment may result. In such cases the sediment should be separated from the supernatant liquid and fumed with 10–15 ml. of sulphuric acid ; the residue should then be added to the decanted liquid and, after boiling, dissolution should be complete.

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[1] Brooks, R. R., Ahrens, L. H., and Taylor, S. R., *Geochim. et Cosmochim. Acta*, **18**, 3 (1960).
[2] Edge, R. A., and Ahrens, L. H. (In the press).

METALLURGY

Corrosion Inhibition and the Zero Charge Potential of Metals

The electrode potential of a mild steel specimen immersed in sodium chloride solutions has been measured in conditions of very rapid aeration produced by bubbling air vigorously over the specimen. Corrosion is rapid, but the potential does not fall to the values observed in conditions of moderate aeration. It rises to a maximum value that can be maintained for an appreciable time even though corrosion continues at a rapid rate. (This maximum potential must be related to the maximum rate of cathodic depolarization as limited by the rate of diffusion of oxygen across the boundary layer on the surface of the metal.) The relationships between potential, chloride concentration and surface preparation of the steel are specific and reproducible and will be described elsewhere. At lower rates of aeration, lower (less-positive) steady potentials can similarly be maintained. This method of potential stabilization was used to test the theory put forward by De[1] for inhibition in neutral and acid solution. De suggested that inhibitive anions are adsorbed preferentially on metal/solution interfaces that are positive to the zero charge potential (potential of electrocapillary maximum) of the metal, that is, he suggested, on cathodic surfaces in neutral solution. Evidence for such preferential adsorption has been found by Hoar and Holliday[2], by Antropov *et al.*[3] and others for inhibition in acid solutions ; but so far as I am aware no direct evidence exists for its validity in neutral solutions.

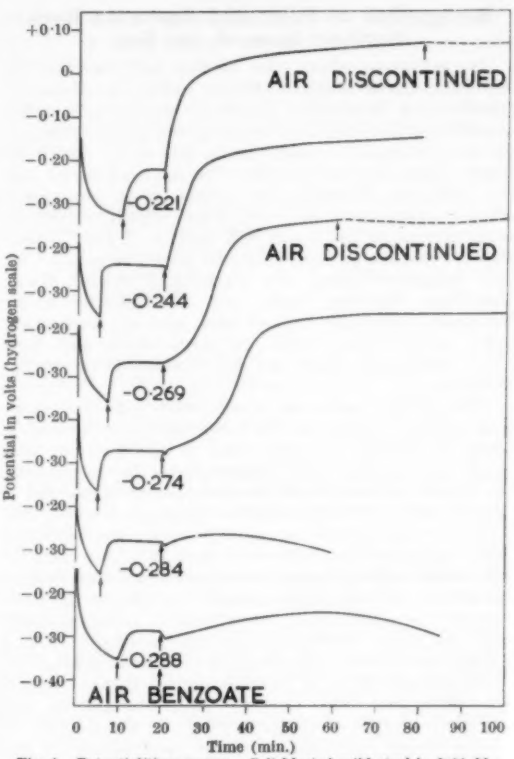

Fig. 1. Potential/time curves. Grit-blasted mild steel in 0·01 *M* sodium chloride

Grit-blasted specimens were suspended in 0·01 *M* sodium chloride (at approximately 20° C.), and after the potential had fallen to approximately −0·36 V. (hydrogen scale), which happens quickly in these conditions, a slow stream of air was passed through the solution (not over the specimen) at such a rate as to cause the specimen to attain a steady pre-determined potential. After 20-min. total immersion of the specimen, sodium benzoate was added, in sufficient amount to make the resulting concentration 0·1 *M*. From Fig. 1 it is seen that at potentials above −0·28 V. benzoate will induce inhibition ; the higher the potential is above this value, the more readily does inhibition occur. Below this potential it will not induce inhibition. The conditions in all the

solutions are precisely similar, the only variant being the potential reached by the specimen at the moment of addition of the benzoate. As the conductivity of the solution is high, the potentials of anodic and cathodic surfaces must be close together; hence adsorption presumably occurs over the whole surface irrespective of the anodic or cathodic nature of local areas.

If this hypothesis is correct, then the zero charge potential of steel in these solutions (0·01 *M* sodium chloride at *p*H 6) is approximately −0·28 V. (hydrogen scale). (Ayazyan[4] reports a value of −0·37 V. for iron in 0·001 *N* sulphuric acid.) Some corroboration that this value does correspond to the zero charge potential is provided by the growth of interference colour films on cathodically polarized surfaces; under similar conditions of *p*H, deposition of the positively charged colloidal corrosion product takes place at potentials just below this[5].

These results can now be used to interpret inhibitor mechanism in solutions that are in equilibrium with air, but not vigorously aerated. The primary function of oxygen (in all solutions of non-oxidizing inhibitors) is clearly to maintain the potential of the specimen above the zero charge potential for a sufficient time to enable the inhibitive anion to become adsorbed. De[1] showed that this took a measurable time (of the order of seconds) in the case of chromate ions. Upon immersion of the specimen, the potential will have a tendency to fall rapidly, the more so the higher the chloride concentration of the solution and the more reactive the metal surface (for example, grit-blasted in comparison with emeried); if the anion can become adsorbed quickly enough the fall in potential will be arrested and inhibition will ensue. For example, it was found that grit-blasted steel, in a stagnant solution of the same final composition as that of the solutions already used (0·01 *M* sodium chloride + 0·1 *M* sodium benzoate), remained protected, but only just; the potential fell slightly during the first 5 min. of immersion, then recovered. The 'fate' of the specimen will thus be decided by the relative speeds of two changes: the fall in potential (resulting from oxygen consumption and a decrease in anodic polarization) and the adsorption of anions. Aeration is not necessary in solutions of nitrite or chromate (or other oxidizing inhibitors), because these substances can raise the potential of the steel sufficiently to allow their own anions to become adsorbed.

This hypothesis explains several facts (hitherto unexplained) relating to inhibition by sodium benzoate. (*a*) Benzoate alone will not protect a badly rusted surface in stagnant conditions; in practice, the situation might be saved by a vigorous aeration 'boost'. (*b*) If benzoate is present in insufficient concentration to give inhibition, then general, but not localized, corrosion occurs. This may be ascribed to the utilization of the nearby oxygen in the early stages of breakdown, followed by a fall in potential, with consequent desorption of the rest of the benzoate anions. In highly aerated conditions, such as those produced by rapid movement, benzoate might no longer be a 'safe' inhibitor. With chromate or nitrite, even in stagnant solutions, the potential (except in case of excess chloride) tends to remain above the zero point owing to the oxidizing properties of these solutions. (*c*) The behaviour of steel in benzoate solution, when coupled to zinc or noble metals, was studied by Gilbert[7], who showed that (1) if steel and zinc were immersed separately in benzoate for 43 hr. and then connected, the potential of the couple was −0·21 V. (hydrogen scale), and the steel remained bright; (2) if steel and zinc in benzoate were connected from the outset, the steady potential of the couple remained in the region of −0·45 to −0·37 V.; that of galvanized steel remained at −0·35 V.; rusting of the steel took place in both cases; (3) no rusting occurred when steel was connected in benzoate solution to platinum, copper, or platinum with an externally applied current of 35 μamp., with the steel (1·5 cm. × 1·4 cm.) as anode; heavy rusting occurred if the current was reversed and the steel made the cathode. The potentials were not given but the results agree qualitatively with the hypothesis.

The potential of an immersed metal, relative to its zero charge potential, is clearly of fundamental importance in determining whether inhibition, due to adsorption of ions, is possible; in particular, a potential positive to the zero point is essential for inhibition by anions. Factors that determine this potential include the surface condition of the metal, the concentration of aggressive ions in the environment and the presence of oxygen or other cathodic depolarizers, contact with other metals or the passage of current.

Thanks are due to Dr. F. Wormwell for his interest and advice. This communication is published by permission of the Director of the National Chemical Laboratory.

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Feb. 8.

[1] De, C. P., *Nature*, **180**, 803 (1957).
[2] Hoar, T. P., and Holliday, R. D., *J. App. Chem.*, **3**, 502 (1953).
[3] Antropov, L. I., Grigor'ev, V. P., and Petrenko, A. T., *Zh. prikl. Khim.*, **31**, 1497 (1958).
[4] Ayazyan, E. O., *Doklady Akad. Nauk. S.S.S.R.*, **100**, 473 (*Chem. Abst.*, **49**, 11533a (1955)).
[5] "Chemistry Research 1958", 30 (H.M.S.O., London, 1959) and unpublished results.
[6] Brasher, D. M., and De, C. P., *Nature*, **180**, 28 (1957).
[7] Gilbert, P. T., and Hadden, S. E., *J. App. Chem.*, **3**, 545 (1953).

Variation of Magnetic Moment with Composition in the Iron–Vanadium Sigma Phase

SINCE the structure of the sigma phase was established by Bergman and Shoemaker[1], it has been found in many binary alloys of transition elements (see, for example, Haworth and Hume-Rothery[2]). Where the phase boundaries have been established, the width of the range of composition varies from less than 1 per cent in the molybdenum–cobalt system to about 20 per cent in the iron–vanadium system. On the basis of the positions of the phase in various binary alloy phase diagrams, it has been suggested that the sigma phase is some kind of electron compound (see, for example, Kasper[3]). Greenfield and Beck[4] concluded that while the sigma phase might be associated with a particular average number of vacancies in electronic '*d*' states per atom, no self-consistent scheme could be developed to reconcile both theory and experiment. Nevertheless, the possibility that '*d*' state vacancies might play a part in stabilizing the structure suggests that magnetic evidence would prove of value. Consequently, in a survey of many binary and ternary sigma phase alloys, Nevitt and Beck[5] concluded that all were ferromagnetic, having Curie points in the temperature range 50–200° K. No measurements had been made,

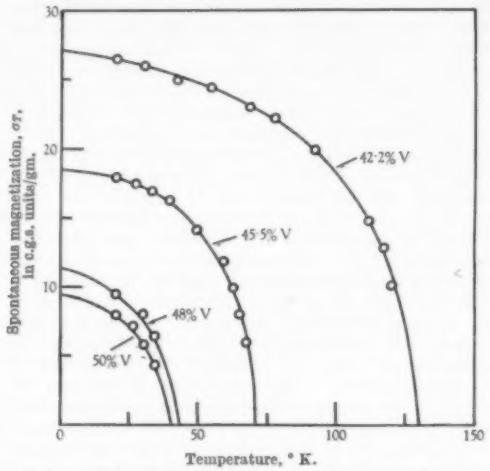

Fig. 1. Variation of spontaneous magnetization, σ_T, with temperature for the four iron–vanadium sigma phase alloys

however, of the mean magnetic moment per atom (p_B) of any ferromagnetic sigma phase, or of its variation, if any, with composition, and so the present work was undertaken, the iron–vanadium system being chosen because of the comparatively wide range of composition over which the sigma phase extends.

The alloys were prepared by melting together appropriate amounts of iron and vanadium in an electric arc furnace under an atmosphere of argon at a pressure of about 40 cm. of mercury. The iron used was B.I.S.R.A. iron of 99·96 per cent purity, and the vanadium was supplied by New Metals and Chemicals, Ltd., and was of 99·5 per cent purity. The sigma phase was precipitated by heating for four days *in vacuo* at 1,050° C., the presence of the phase then being confirmed by X-ray diffraction. Measurements of the magnetization were then made at temperatures between 20° K. and the Curie point and in applied magnetic fields of between 4 and 16 kilo-oersteds, using a Sucksmith ring balance[6]. The linear part of the magnetization curves at the constant temperature, T, were extrapolated to zero field to determine the spontaneous magnetization at that temperature, σ_T. The spontaneous magnetization at absolute zero, σ_0, and the Curie point, θ_f, were deduced using methods which have become standard in this laboratory (see Crangle[7]). The results are shown in Fig. 1 and Table 1, and from them it can be seen that there is a decrease in magnetic moment and Curie point with increasing vanadium content. A linear extrapolation of the curve of magnetic moment against composition goes to zero moment at a composition corresponding to about 53 per cent vanadium although the composition limit of the sigma phase is about 57 per cent vanadium. The Curie points are considerably lower than that of the α-phase of the same composition as deduced from Fallot[8], so ruling out the possibility that the ferromagnetism is due to retained alpha phase.

Table 1

Composition percentage V (by atoms)	σ_0 (c.g.s. units/gm.)	Curie point (° K.)	p_B (Bohr magnetons)
42·2 ± 0·5	27·1	130	0·261
45·5 ± 0·1	18·4	72	0·177
48·0	11·5	44	0·110
50 ± 2	9·5	40	0·091

There would seem to be two possible interpretations of the results.

If the magnetic moment of the sigma phase is due to a net unbalance of positive and negative spins in a common '*d*' band, then there is a redistribution of spins in the band as the composition changes, leading to a balance of spins at 53 per cent vanadium. The phase does not, however, cease to be stable at this point.

The alternative interpretation is that there is a ferri-magnetic arrangement of magnetic ions in the structure which alters with composition. This would seem to be the more probable interpretation, particularly in view of the fact that the curves of inverse susceptibility against temperature above the Curie point are slightly concave to the temperature axis.

The compositions quoted in Table 1 are based on the amount of each constituent present before melting, and the errors estimated from the loss in weight during melting.

Further work is in progress on the iron–vanadium system, and also on other sigma phase alloys.

It is a pleasure to record my thanks to Prof. W. Sucksmith for his advice and for his continued interest in the work.

The alloy quoted as containing 48 per cent vanadium was kindly supplied by Dr. B. R. Coles.

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[1] Bergman, G., and Shoemaker, D. P., *Acta Cryst.*, **7**, 857 (1955).
[2] Haworth, C. W., and Hume-Rothery, W., *Phil. Mag.*, **3**, No. 33, 1013 (1958).
[3] Kasper, J. S., "Theory of Alloy Phases" (A.S.M.), 264.
[4] Greenfield, P., and Beck, P. A., *J. Metals*, **8**, 265 (1956).
[5] Nevitt, M. V., and Beck, P. A., *Trans. Amer. Inst. Mining and Metallurgical Eng.*, **203**, 669 (1955).
[6] Sucksmith, W., *Proc. Roy. Soc.*, A, **170**, 551 (1939).
[7] Crangle, J., *Phil. Mag.*, **46**, 499 (1955).
[8] Fallot, M., thesis, Strasbourg (1935).

CHEMISTRY

Conductivity of Aqueous Sodium Stannate Solution

In view of the attention which has recently been paid to the chemistry of silicates and germanates[1,2] the following results obtained during an investigation of the properties of sodium stannate solutions are of interest.

When sodium stannate, prepared by Zocher's method[3], is dissolved in water at 25·0° C. there is an immediate increase in *p*H, after which the solution becomes slowly more alkaline, reaching a steady limiting value after about one week. In order to follow this reaction the variation in conductivity with time was measured using an electrodeless capacity-coupled cell[4]. Over the whole of the concentration-range studied the specific conductance increased regularly with time and reached a limiting value after seven days. The specific and equivalent conductances at zero time were obtained by graphical extrapolation of the conductivity-time plots; the results are given in Table 1.

Table 1. Equivalent Conductance of Sodium Stannate Solutions at Zero Time

Molar concentration sodium stannate	0	$1{\cdot}035 \times 10^{-4}$	$1{\cdot}179 \times 10^{-4}$	$1{\cdot}735 \times 10^{-4}$	$2{\cdot}639 \times 10^{-4}$
Equivalent conductance (ohm^{-1} $cm.^2$)	111·8	110·7	109·0	107·0	106·4
Molar concentration sodium stannate	$5{\cdot}485 \times 10^{-4}$	$7{\cdot}018 \times 10^{-4}$	$1{\cdot}051 \times 10^{-3}$	$1{\cdot}527 \times 10^{-3}$	$3{\cdot}553 \times 10^{-3}$
Equivalent conductance (ohm^{-1} $cm.^2$)	105·0	103·1	100·3	99·5	93·9
Molar concentration sodium stannate	$4{\cdot}896 \times 10^{-3}$	$5{\cdot}216 \times 10^{-3}$	$6{\cdot}993 \times 10^{-3}$	$7{\cdot}564 \times 10^{-3}$	$1{\cdot}246 \times 10^{-2}$
Equivalent conductance (ohm^{-1} $cm.^2$)	92·7	91·4	91·1	88·6	85·6

The equivalent conductivity at infinite dilution, 111·8, was obtained from a Kohlrausch plot; on correction for the effect of hydrolysis has been made. The difference between this value and the value of 156 obtained by Bellucci and Parravano[5] for potassium stannate is greater than the theoretical one. Bellucci and Parravano, however, only noted an increase in conductivity with time in their most concentrated solution, so it is possible that their potassium stannate was impure.

The increase in conductivity with time and the final attainment of a limiting value shows that the species initially present are reacting at a measurable rate. By means of *p*H measurements it has been shown that for approximately the first 70 per cent of the reaction the increase in specific conductivity is a direct measure of the increase in hydroxyl ion concentration of the solution. The rate of production of hydroxyl ions is a first or pseudo-first order reaction with a rate constant of $5{\cdot}3 \times 10^{-3}$ $hr.^{-1}$. The hydrolysis of sodium stannate is thus seen to occur in at least two stages: first, a fast reaction, typified by the immediate alkaline reaction of a freshly prepared stannate solution, and, secondly, by a slow first or pseudo-first order formation of more hydroxyl ions. Unfortunately, there are insufficient data available at present to make a more detailed mechanistic analysis of these reactions; but in view of the fact that both silicates and germanates form polynuclear hydrolysis products it seems likely that a similar explanation would apply in the case of stannate solutions.

I wish to thank the Superintendent of the Admiralty Materials Laboratory for permission to publish this work.

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[1] Igri, N., Abstracts International Conference on Co-ordination Chemistry, London, 1959.
[2] Everest, D. A., and Harrison, J. C., *J. Chem. Soc.*, 2178 (1959).
[3] Zocher, H., *Z. anorg. Chem.*, **112**, 1 (1920).
[4] Bromley, D. E., and Good, P. T., *J. Sci. Instr.*, **36**, 326 (1959).
[5] Bellucci, J., and Parravano, N., *Atti. Accad. Lincei*, **13**, 307 (1904).

Toxicity of Plastics

In view of the ever-increasing use of plastics in contact with foodstuffs and like materials and the enormous amount of work done to establish their suitability for such uses, second thoughts on materials condemned without actual test might be advisable. In this respect I would like to direct attention to the results of a series of tests carried out in the laboratories of Bakelite, Limited.

We were asked to investigate the leaching-out of lead compounds from lead-stabilized polyvinyl chloride compositions in contact with a constant volume of hard and soft water. These tests were carried out by storing 6 litres of water at room temperature in tanks 18 in. × 12 in. × 5 in. deep made from the sample material by welding press-polished sheet and, after a period of 18 months contact with the water, determining the total lead content of the waters. It was shown that, with a compound stabilized with 3·77 per cent basic lead phosphite and lubricated with 0·94 per cent basic lead stearate, 0·5 part per million lead were extracted by soft water and no lead by hard water; while a compound, containing only 3·81 per cent dibasic lead phosphite, had none of its lead extracted by either hard or soft water. Thus it appears that only some types of lead compound are extracted from these compositions by water.

Although the test specimens employed and the test methods used are not those specified by the British Plastics Federation in its Toxicity Report No. 40, dated 1958, nevertheless, it is felt that the results are of considerable interest to the industry as a whole. If the total amount of lead extracted from a sample over a period of 18 months is only 0·5 part per million, then surely such a material would be satisfactory for use in pipes in contact with flowing water. In the event of water being stagnant in the pipes up to 18 months it could only produce a lead concentration of 0·5 part per million. On the other hand, formulations can be made which contain lead compounds, for example, lead phosphite, from which no lead is extracted either by hard or soft water. These results could, therefore, be considered good evidence in favour of allowing a formulation containing lead compounds to be employed in contact with water in conformity with Continental practice.

In conclusion, I suggest that polyvinyl chloride compositions should not necessarily be condemned as being toxic just because they contain toxic substances, but should be considered in the light of the applications for which they are intended to be used.

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Separation Factors in Gas Chromatography

It is generally accepted among those using gas chromatography that for most separations the use of lower temperatures increases the separation factor, though, usually owing to the time taken at low temperatures, there is a limit to the use to which this effect can be put. Capillary columns can, however, be made extremely fast in their operation, and hence low temperatures can be used for separations which normally require temperatures at least 70° C. higher.

The classically difficult separation in gas chromatography is that of *meta*- and *para*-xylene. Although nowadays this separation is relatively commonplace owing to the use of capillary columns and selective stationary phases, an interesting separation was made in the course of routine testing at our laboratory which illustrates the effect of low-temperature operation. In this case, the stationary phase was a non-

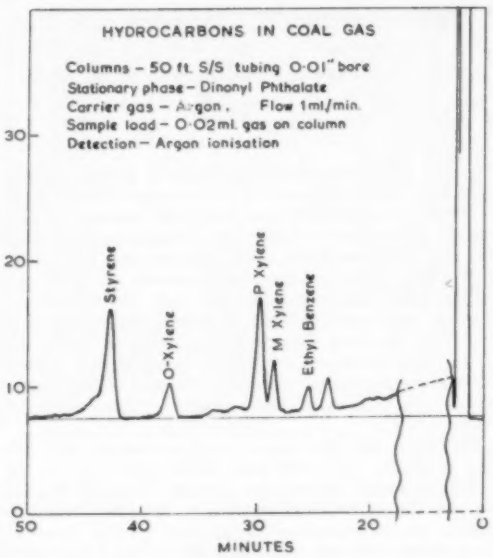

Fig. 1

selective one, dinonyl phthalate. At room temperature, these two isomers are easily separated, the separation factor being 1·05. A typical chromatogram is shown in Fig. 1. No separation is obtained at 80° C., although the plate number remains the same. It is clear that the principal separating factor with these two substances is their difference in vapour pressure, and that the difference is much greater at room temperature than it is at more elevated ones.

It is probable that the large increase in separation factor towards these really low temperatures is a general one.

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BIOCHEMISTRY

Hepatotoxic Activity of Semi-Synthetic Analogues of Pyrrolizidine Alkaloids

Many of the natural pyrrolizidine alkaloids isolated from various plants are not only acutely hepatotoxic, but are also able to produce subacute and chronic liver lesions in rats when given in an appropriate single dose. The most striking feature of the lesions are the cytological abnormalities of the liver parenchymal cells, suggesting that the alkaloids may be interphase mitotic poisons[1]. The hepatotoxic activity of these alkaloids, especially in relation to their basic moieties, has been discussed[2]. Though their structures vary greatly, yet one feature is common to all, namely, the branching of the carbon-chain of their acidic moieties, the necic acids.

Such structures could in the course of the metabolic degradation in the animal body yield isoprenoid units, or their homologues. In view of the finding that isoprenoid units, derived from mevalonic acid (3 : 5-dihydroxy-3-methyl-valeric acid), are incorporated *in toto* into squalene, the precursor of cholesterol[3], and probably into steroidal hormones, and other polyprenoids, it is conceivable that the hepatotoxic pyrrolizidine alkaloids interfere in some way with the biosynthesis of some such vital cell constituents. The fact that young, immature animals are more susceptible than adults, and males more than females, to the action of pyrrolizidine alkaloids[4] suggests also that steroidal hormones may be implicated.

Table I. Hepatotoxic Activity in Rats

Compound	Dose range (mgm./kgm. body-weight)	Cytological abnormalities of liver
Retronecine hydrochloric acid	600–1,000	–
Monocrotalic acid lactone (sodium salt)	400–1,300	–
Tiglic acid (sodium salt)	60–1,670	–
Tiglic amide	140– 600	–
Senecioic acid (sodium salt)	400–2,500	–
Senecioic amide	170– 500	–
Di-*n*-butyryl retronecine	175–1,300	–
Di-*n*-valeryl-retronecine	480– 730	–
Di-*iso*-valeryl-retronecine	300– 830	++
Di-tigloyl-retronecine	100– 500	++
Di-senecioyl-retronecine	200– 525	+++

Yet, the free necic acids obtained by hydrolysis of the natural alkaloids are not hepatotoxic, nor are synthetic acids with an isoprenoid structure such as senecioic or tiglic acids, or their amides (Table 1).

It was thought desirable to try to prepare semi-synthetic analogues of pyrrolizidine alkaloids with a series of related straight and branched-chain, monocarboxylic acids, in order to evaluate the significance of the branching of the carbon chain of the acidic moieties in relation to their hepatotoxic activity.

The amino-alcohol used for this purpose was natural retronecine, obtained by hydrolysis of monocrotaline (the main alkaloid of *Crotalaria retusa* L.), according to the procedure of Adams and Rogers[5]. This was esterified by the action of the respective acid chlorides, yielding mainly diesters. These were oily liquids, and were purified via their crystalline picrates. The preparation of di-senecioyl-retronecine may serve as an example. Retronecine hydrochloride was heated with an excess of redistilled senecioyl chloride, on a water-bath for 15 min.; the excess of the chloride was decomposed with alkali and the ester, extracted with ether, was isolated as a crystalline picrate, yellow needles, m.p. 115° C., from ethanol (found : C, 52·9 ; H, 5·24 ; N, 10·3 per cent : $C_{24}H_{28}N_4O_{11}$ requires C, 52·6 ; H, 5·1 ; N, 10·2 per cent). The diester, a colourless viscous oil, was recovered from the picrate by shaking with aqueous ammonia and extracting with ether. It formed a crystalline hydrochloride, colourless needles from acetone-ether, m.p. 126° C. (found : C, 60·5 ; H, 7·37 ; N, 4·3 per cent : $C_{18}H_{26}NO_4Cl$ requires C, 60·7 ; H, 7·3 ; N, 3·9 per cent).

For biological testing the respective compounds were dissolved, by the addition of equimolecular amounts of dilute hydrochloric acid, or in water when present as hydrochlorides, and various, single doses were given by stomach tube or by intraperitoneal injections to weanling Wistar rats. The animals which did not die within a few days were allowed to survive for at least 1–2 months, before they were killed and examined. The livers, lungs and sometimes other organs were then fixed and processed in the usual way for microscopic examination. The presence of significant numbers of characteristic large parenchymal liver cells was taken as evidence of hepatotoxic activity.

The diesters, when tested by intraperitoneal injections, caused convulsions and death of the rats, with

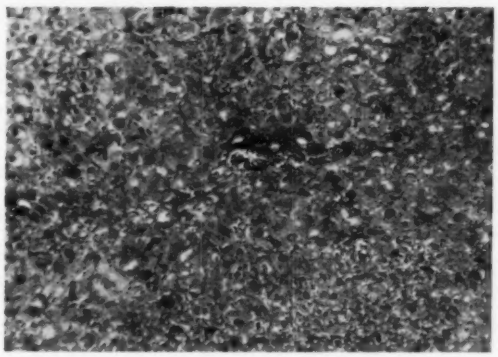

Fig. 1. Liver of a male rat, killed 3 months after a single dose of di-senecioyl-retronecine, 450 mgm./kgm. body-weight, showing great variation in the size of parenchymal cells and infiltration of portal areas (hæmatoxylin and eosin, × 100)

accompanying lung œdema, after doses much smaller than (about 1/5) those which produced similar effects by the oral route. Indeed, only some of the compounds were given in high enough oral doses to induce convulsions, which were then less intense and transitory, so that the animals only died a few days after the treatment.

Subacute liver lesions and cytological abnormalities indistinguishable from those seen after the natural pyrrolizidine alkaloids were produced by the diesters of retronecine with tiglic and senecioic acids, administered orally (Fig. 1). The doses required to produce these lesions were of the order 250–500 mgm./kgm. body-weight (Table 1). These doses are higher than those of the natural alkaloids, whether the cyclic or the open esters[2], which produce similar lesions. This may be due to the use of the synthetic products as such, without trying to resolve them into optically active isomers.

The liver lesions produced by di-*iso*-valeryl-retronecine were comparable, but no evidence of abnormalities were seen in the livers of rats given di-*n*-butyryl-, and di-*n*-valeryl-retronecine, even after doses as high as 1,300 mgm. and 730 mgm./kgm. body-weight, respectively.

These results are consistent with the view that branching of the carbon-chain of the acidic moieties is necessary for hepatotoxic activity of this class of compounds. With the possibility now open for the synthesis of appropriately labelled hepatotoxic analogues of pyrrolizidine alkaloids, further work on the mechanism of their toxic action can be pursued.

The experimental details of this work will be published elsewhere. We wish to thank Dr. C. D. Adams, Department of Botany, Achimota, and Dr. R. A. Galley, Tropical Products Institute, London, for their kind help in obtaining large quantities of *Crotalaria retusa* from Ghana, as a source of monocrotaline, and Dr. J. M. Barnes for his stimulating interest.

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[1] Schoental, R., and Magee, P. N., *J. Path. Bact.*, **78**, 471 (1959).
[2] Schoental, R., *Nature*, **179**, 361 (1957).
[3] Cornforth, J. W., Cornforth, R. H., Popjak, G., and Gore Youhotsky, I., *Biochem. J.*, **69**, 146 (1958).
[4] Schoental, R., *J. Path. Bact.*, **77**, 485 (1959).
[5] Adams, R., and Rogers, E. F., *J. Amer. Chem. Soc.*, **61**, 2815 (1939).

Formation of L-Xylosone from Ascorbic Acid

During a study of the carbonyl compounds produced during cider fermentations an immediate precipitate (*A*) was obtained at room temperature on addition of dinitrophenylhydrazine to a sulphited cider (that is, a cider obtained from a juice to which 150 parts per million sulphur dioxide had been added after milling and pressing the fruit). A similar precipitate was formed in a sulphited Bramley Seedling juice which had failed to ferment, but no appreciable precipitate was noted in non-sulphited fermentations. The derivative (*A*) on a paper chromatogram gave a blue colour with sodium hydroxide solution which indicated the possibility of osazone formation. Owing to the difficulty of purifying the 2 : 4-dinitrophenylhydrazine derivative, the phenylhydrazine derivative (*B*) was prepared from a sulphited cider at room temperature.

Paper chromatography, that is, examination of derivative *B* by running on the same untreated No. 1 Whatman paper and on borate-treated paper with the phenylosazones of glucose, xylose and arabinose in dioxane–heptane–water (2 : 1 : 1), suggested that *B* was xylosazone.

Table 1

	R (arabinosazone) values	
	Untreated paper	Borate-treated paper
L-Arabinosazone	1·00	1·00
D-Xylosazone	1·00	0·25
Isolated derivative (*B*)	1·00	0·25
D-Glucosazone	0·37	0·00

Derivative *B* was purified by elution from an alumina column[1] with alcohol–water (9 : 1) after washing with benzene, benzene–ether (1 : 1) and ether. After evaporating to dryness *B* was recrystallized from methanol–water and had a melting point 164–165°, identical with that of D-xylosazone. A mixed m.p. of *B* and D-xylosazone gave m.p. 196°, which indicated that compound *B* was L-xylosazone. The m.p. of L-xylosazone made from a commercial sample of L-xylose was 168–169° and a mixed m.p. of this derivative with compound *B* gave 166–167°. Compound *B* was, therefore, identified as L-xylosazone and the substance present in the cider as L-xylosone.

The origin of this substance is of some interest. Recent work has shown that sugars may be oxidized to osones by the action of bisulphite[2]. However, the xylose present in the cider-apple juices is D-xylose (shown by m.p. and mixed m.p. of isolated xylosazone and of an authentic specimen). A more likely source of L-xylosone is L-ascorbic acid, which has been shown to occur in appreciable amounts in cider apple juices[3]. During the milling and pressing processes prior to the addition of sulphur dioxide it would be expected that much L-ascorbic acid would be converted to L-dehydroascorbic acid, also dehydroascorbic acid may be present in amounts similar to ascorbic acid in apples[4]. Opening up of the lactone ring followed by decarboxylation would yield L-xylosone.

A sample of ascorbic acid was oxidized to dehydroascorbic acid with iodine solution and just decolorized with sulphur dioxide solution. Sufficient sulphur dioxide solution was then added to combine with the carbonyl groups. After standing three days at room temperature the solution on addition of 2 : 4-dinitrophenylhydrazine rapidly gave a precipitate in the cold. A larger precipitate was obtained after boiling

the solution for 30 min. Addition of phenylhydrazine and acetic acid gave an osazone in the cold with m.p. 164–165° unchanged on mixing with L-xylosazone isolated from cider. Standing or heating L-dehydroascorbic acid with sulphur dioxide therefore formed L-xylosone.

Previous work has shown that ascorbic acid is oxidized by perinaphthindan-2 : 3 : 4 trione hydrate at 45° to dehydroascorbic acid, which then undergoes hydrolysis and decarboxylation to yield L-xylosone[5]. Similarly, oxidation with ninhydrin[6] and alloxan[7] was followed by decarboxylation with the probable formation of L-xylosone. The formation of L-xylosone from dehydroascorbic acid in the presence of sulphur dioxide is of interest in view of the high proportion of dehydroascorbic to ascorbic acid in apples, pears and cherries[4] and of the use of sulphur dioxide as a preservative in other food products.

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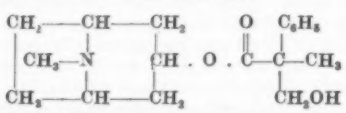
[1] Barry, V. C., and Mitchell, P. W. D., *J. Chem. Soc.*, 4020 (1954).
[2] Ingles, D. L., *Chem. and Indust.*, 1045 (1959).
[3] Kieser, M. E., and Pollard, A., *Nature*, **159**, 65 (1947).
[4] Koch, J., and Bretthauer, G., *Landwirtsch. Forsch.*, **9**, 51 (1956).
[5] Moubasher, R., *J. Biol. Chem.*, **176**, 529 (1948).
[6] West, E. S., and Rinehart, R. E., *J. Biol. Chem.*, **146**, 105 (1942).
[7] Nath, M. C., and Bhattathiry, E. P. M., *Nature*, **176**, 787 (1958).

Anticholinergic Activity of Tropine-α-Methyltropate

The synthesis of the new α-methyltropic ester of tropine and the isolation of the two optically active isomers has been recently described by Melone, Vecchi, Pagani and Testa[1].

These compounds (DL, (+)- and (−)-tropine-α-methyltropate) were supposed to display an anticholinergic activity possibly different from atropine itself (especially as far as the duration of action is concerned). In fact, the above-quoted authors, as well as Foster and Ing (who attempted to synthesize the same compounds[2]), had suggested that the esters of α-methyltropic acid, being very difficult to hydrolyse, should lead to quite stable compounds. Moreover, the natural occurring (−)-atropine, practically responsible for most of the action of atropine[3], very easily undergoes spontaneous racemization, with loss of activity. On the contrary, α-methyltropic acid and its esters, lacking the hydrogen in α- to the carboxyl group, has no tendency to racemize. This fact appeared of particular interest, as it was clear from the first experiences that only the (−)-isomer of tropine α-methyltropate possesses a significant anticholinergic activity.



Tropine-α-methyltropate

The stability of tropine α-methyltropate was believed to exercise an influence not only on the degree but also upon the duration of the pharmacological effects, as it is well known that the natural alkaloid, atropine, is destroyed by mammalian tissues, mostly through enzymatic hydrolysis of the ester to tropine (or tropanol) and tropic acid[4].

This communication concerns the results of a preliminary pharmacological investigation, carried out on (−)- and (+)-tropine α-methyltropate hydrochlorides.

The anticholinergic activity of the two compounds was evaluated and compared with that of atropine sulphate, on isolated rat gut and on the cardiovascular response of the dog to intravenously injected acetylcholine.

Acute intravenous toxicity was studied in *CF*-1 mice.

In isolated rat intestine the products were added to the oxygenated Tyrode solution of the bath chamber in fixed volume of 0·2 ml., 1 min. before the introduction of acetylcholine chloride. The recorded effects were evaluated as reduction of the contractile response to acetylcholine.

In preliminary tests it was demonstrated that (−)- and (+)-isomers and atropine display an anticholinergic activity qualitatively similar.

The concentration/activity curves of the three drugs were found to be parallel, thus allowing the estimation of relative potency. Three comparisons were carried out: between atropine sulphate and the (−)-α-methyl derivative hydrochloride; between atropine sulphate and the (+)-α-methyl derivative hydrochloride; and finally between the two optical antipodes. By calculating the ratios between equally effective concentrations (that is, the concentrations effective in reducing to 50 per cent the response of acetylcholine afterwards administered) the following potency ratio resulted:

Atropine/(−)-methylatropine (No. of experiments, 8)	1–2
Atropine/(+)-methylatropine (No. of experiments, 7)	1–0·04
(−)-Methylatropine/(+)-methylatropine (No. of experiments, 9)	1–0·02

The average effective concentration of atropine sulphate was found to be within a range of 0·016–0·012 μgm./ml. The average effective concentration of (+)-α-methylatropine was found to be 0·36–0·22 μgm./ml., while for (−)-α-methylatropine it was found to be 0·0074–0·0038 μgm./ml.

In dogs anæsthetized with sodium pentobarbitone the ability of (−)-methylatropine and atropine sulphate to prevent the fall in blood pressure caused by intravenous administration of 2 μgm. of acetylcholine was compared and the duration of the inhibitory effect especially considered. Each of the two products was injected intravenously in doses of 5 μgm./kgm. and acetylcholine administered before and 2, 6, 16, 26, 36 and 46 min. after the dosage until the fall in blood pressure produced by acetylcholine was returned to pre-treatment level. Six dogs were employed for these experiments. Three of them received the two products alternately. The three other dogs received the two drugs on the basis of a random schedule. The dose-level of 5 μgm./kgm. was chosen after preliminary assays that demonstrated this to be approximately the average effective dose for the two drugs.

In Table 1 the results of these experiments are briefly summarized.

The *LD*50 of (−)-atropine-α-methyltropate after intravenous administration in mice was found to be 102 mgm./kgm. (confidence limits $P = 0{\cdot}05$: 89·57–116·3) (according to Litchfield and Wilcoxon[5]), while for atropine the intravenous *LD*50 is 76·7 mgm./kgm. (confidence limits: 71·8–81·9). Also acute toxicity of both drugs in mice after intravenous administration gave the same result.

Table 1

Compound	No. of experiments	Dose (μgm./kgm.)	Average inhibition (per cent) of the depressor effect of acetylcholine (2·0 μgm./kgm.) after 2 min.	6 min.	16 min.	26 min.	36 min.	46 min.
Atropine sulphate	18	5	81·00 ± 7·62	59·50 ± 6·13	41·16 ± 7·32	27·83 ± 8·48	13·20 ± 7·33	3·80 ± 1·64
(−)-Methyltropine	15	5	81·20 ± 5·58	51·60 ± 10·4	34·00 ± 7·72	23·25 ± 9·24	13·00 ± 8·19	10·75 ± 8·29

Our preliminary experiments indicate that between the two optical antipodes of the new derivative tropine-α-methyltropate, the (−)-isomer shows *in vitro* a cholinergic blocking activity about fifty times greater than the corresponding (+)-isomer. This difference is substantially of the same order as between the optical isomers of the naturally occurring alkaloid atropine. Bovet and Bovet-Nitti[3] report that *l*-hyosciamine is ten to one hundred times more active than the *d*-isomer, according to different evaluations.

In *in vitro* experiments (−)-atropine-α-methyltropate hydrochloride shows an anticholinergic activity twice as great as atropine sulphate. In the dog, however, atropine sulphate and (−)-tropine-α-methyltropate hydrochloride were found equivalent in their ability to prevent the depressor effect of acetylcholine on blood pressure. Although there is no experimental evidence to support the hypothesis that the great resistance of (−)-atropine-α-methyltropate to hydrolysing agents could implicate a greater duration of pharmacological activity, the preliminary results herein reported seem to indicate that (−)-tropine-α-methyltropate is a drug of potentially practical value.

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[1] Melone, G., Vecchi, A., Pagani, G., and Testa, E., *J. Org. Chem.* (in the press).
[2] Foster, R., and Ing, H. R., *J. Chem. Soc.*, 938 (1956).
[3] Bovet, D., and Bovet-Nitti, F., "Structure et Activité des Médicaments du Système Nerveux végétatif", 500 (S. Karger, Basle, 1948).
[4] Goodman, L. S., and Gilman, A., "The Pharmacological Basis of Therapeutics", second edit., 551 (The Macmillan Co., New York, 1956).
[5] Litchfield, J. T., and Wilcoxon, F., *J. Pharm. Exp. Therap.*, **96**, 99 (1949).

11α-Hydroxylation of Steroids by *Streptomyces* Species

Extensive studies[1] in the past few years have shown that the ability to transform the steroid molecule is widespread among various micro-organisms, and the types of reaction are numerous. Among them, 11α-hydroxylation is a quite common reaction with fungi[1,2]. More recently, it was found that two strains of bacteria have introduced the hydroxy group in progesterone[3] and Reichstein's[4] compound *S* at the 11α-position. However, with Actinomycetes this 11α-hydroxylation has not been reported, while 11β-hydroxylation of compound *S* by *Streptomyces radiae*[5] has been shown.

In the present communication we wish to report that microbiological hydroxylation of progesterone and Reichstein's compound *S* in the 11α-position and the 6β-position has been carried out by unidentified *Streptomyces* species (*Ac*-103) isolated from soil. In our experiment, *Streptomyces* sp. (*Ac*-103) was incubated in a medium containing 1 per cent glucose, 1 per cent peptone, 1 per cent meat extract and 0·3 per cent sodium chloride. After incubation at 28° for a period of 48 hr. on a reciprocating shaker, 1 gm. of the steroid substrate dissolved in methanol was added to 2 l. of medium and the incubation was continued for 48 hr. The culture filtrate was extracted with ethyl acetate ester and the extracts were concentrated to dryness *in vacuo* at 30°. The residue was redissolved in benzene or ethylene dichloride and the solution was chromatographed on an alumina or 'Florisil'.

When progesterone was used as a substrate in the incubation with this micro-organism, 6β,11α-dihydroxyprogesterone was isolated as the insoluble matter in benzene of the condensed residue of extract. Crystallization of this insoluble matter from acetone afforded 400 mgm. of 6β,11α-dihydroxyprogesterone (I), m.p. 234–240°, $[\alpha]_D$ + 110° (methanol), $\lambda_{max.}^{MeOH}$ 236 mμ (ε = 14,200), $\lambda_{max.}^{KBr}$ 3,420 (OH), 1,695 (20-CO), 1,660 and 1,615 (Δ⁴-3-CO) cm.⁻¹. The diacetate of (I) prepared with acetic anhydride and pyridine melts at 150–153°, $[\alpha]_D$ + 70° (chloroform). The infra-red spectra of these compounds agreed with those of authentic samples. Then, the benzene solution filtrate was chromatographed on alumina and benzene–ether–chloroform gradient elutions were used for the separation of other oxygenated steroids. In the first fractions a small amount of progesterone was obtained, and in the second fractions 6β-hydroxyprogesterone was obtained. The crystallization of the second fractions from acetone gave 100 mgm. of 6β-hydroxyprogesterone (II), m.p. 172–173°, $[\alpha]_D$ + 98° (chloroform), $\lambda_{max.}^{MeOH}$ 236 mμ (ε = 13,700), $\lambda_{max.}^{KBr}$ 3,450 (OH), 1,710 (20-CO), 1,669 and 1,620 (Δ⁴-3-CO) cm.⁻¹.

When Reichstein's compound *S* was used as a steroid substrate, the 6β-hydroxylated product and 11α-hydroxylated product were obtained by column chromatography on 'Florisil' using ethylene dichloride–acetone gradient elutions. The product obtained by recrystallization of the first fractions from acetone was 320 mgm. of 6β,17α,21-trihydroxy-4-pregnene-3,20-dione (III), m.p. 230–235°, $[\alpha]_D$ + 51° (methanol), $\lambda_{max.}^{MeOH}$, 238 mμ (ε = 13,300), $\lambda_{max.}^{KBr}$ 3,420 (OH), 1,710 (20-CO), 1,665 and 1,615 (Δ⁴-3-CO) cm.⁻¹. The 6,21-diacetate melts at 190–195°, $[\alpha]_D$ + 60° (methanol). Crystallization of the second fractions afforded 120 mgm. of 11α,17α,21-trihydroxy 4-pregnene-3,20-dione (IV), m.p. 205–210°, $[\alpha]_D$ + 117° (methanol), $\lambda_{max.}^{MeOH}$, 242 mμ (ε = 14,400), λ_{max}^{KBr} 3,442 (OH), 1,715 (20-CO), 1,653 and 1,615 (Δ⁴-3-CO) cm.⁻¹. The 11,21-diacetate melts at 206–208°, $[\alpha]_D$ + 120° (methanol). The infra-red spectra of these compounds agreed with those of authentic samples.

From this experiment, 11α-hydroxylation of a steroid, which is one of the most important and common reactions among fungi, is carried out equally by Actinomycetes, fungi and bacteria. But, it may be worth mentioning that there is a remarkable difference in bioconversion patterns between Actinomycetes and fungi, for although both *Streptomyces* sp. and fungi, for example *Rhizopus arrhizus*, produced the

same 6β, 11α-dihydroxyprogesterone as a dihydroxylated product from progesterone, they produced a different monohydroxylated product; *Streptomyces* sp. produced 6β-hydroxyprogesterone and, in contrast, *Rhizopus arrhizus* 11α-hydroxyprogesterone. So the enzyme systems of these two micro-organisms would appear to be different.

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[1] Eppstein, S. H., Meister, P. D., Murray, H. C., and Peterson, D. H., *Vitamins and Hormones*, **14**, 359 (1956). Visher, E., and Wettstein, A., "Adv. in Enzymol.", **20**, 237 (1958).

[2] Peterson, D. H., Murray, H. C., Eppstein, S. H., Reineke, L. M., Weintraub, A., Meister, P. D., and Leigh, H. M., *J. Amer. Chem. Soc.*, **74**, 5933 (1952). Fried, J., Thoma, R. W., Gerke, J. R., Herz, J. E., Donin, M. N., and Perlman, D., *J. Amer. Chem. Soc.*, **74**, 3962 (1952).

[3] McAleer, W. J., Jacob, T. A., Turnbull, L. B., Schoenewaldt, E. F., and Stoudt, T. H., *Arch. Biochem. Biophys.*, **73**, 127 (1958).

[4] Sugawara, S., Tsuruta, M., Shirasaka, M., and Nakamura, M., *Arch. Biochem. Biophys.*, **80**, 383 (1959).

[5] Colingsworth, D. R., Karnemoat, J. N., Hanson, F. R., Brunner, M. P., Mann, K. M., and Haines, W. J., *J. Biol. Chem.*, **203**, 807 (1953).

Histochemical Demonstration of a Species-Specific Trypsin-like Enzyme in Mast Cells

During the investigation of a new histochemical substrate, N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride, localization by a simultaneous azo coupling reaction of enzymatic activity to mast cells in human and dog tissues was noted. Recently, Benditt has described the presence of an enzyme in the mast cells of numerous species resembling bovine α-chymotrypsin. This enzyme hydrolyses the histochemical substrate 3-chloroacetyl-2-naphthoic acid anilide[1]. Although N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride has been shown in biochemical systems to be a substrate for trypsin and not for α-chymotrypsin, carboxypeptidase, liver esterase or 'leucine' aminopeptidase[2], it was thought of importance to attempt to define as closely as possible the characteristics of the enzyme in mast cells hydrolysing N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride and to differentiate it from other known mast cell enzymes.

N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride was synthesized by the mixed anhydride method[3]. Coupling of N$^{\alpha}$-benzoyl-N$^{\omega}$-nitro-*l*-arginine with β-naphthylamine was effected in 60 per cent yield, the product melting at 220–3°. The nitro group was removed by hydrogenation with a palladium catalyst in dilute hydrochloric acid to afford optically inactive N$_{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride, m.p. 195–7° (ref. 4). Racemization apparently occurred in the course of activating the benzoylated amino-acid. 3-Chloroacetyl-2-naphthoic acid anilide was synthesized by the method of Gomori[5].

The incubation medium used in the histochemical experiments consisted of 30 mgm. of N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride and 10 mgm. of stabile diazotized 5-methyl-4-(nitro-*p*-tolylazo)-*o*-anisidine[6] (diazotized *o*-aminoazotoluene[7] may also be used) dissolved in 20 ml. of *M*/20 *p*H 7·0 *tris* buffer. Fresh frozen sections of skin or sections

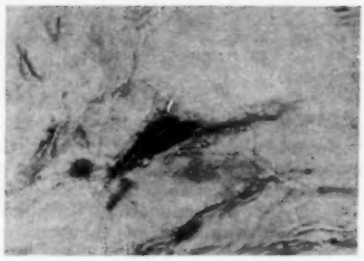

Fig. 1. Localization of trypsin-like enzyme in mast cell of human skin

fixed for 16 hr. in neutral 10 per cent formalin at 4° C. were incubated in the above solution for 20–30 min., placed in a 1 per cent copper sulphate solution for 10 min., washed in running water, and mounted in glycerin gelatin containing several drops of a 1 per cent copper sulphate solution. Dark purple granules of the azo dye were observed only in mast cells (Fig. 1). The possibility of non-specific azo dye or naphthylamine binding by mast cell granules was eliminated when it was observed that tissue sections exposed to either a neutral aqueous suspension of the free naphthylamine (later coupled with diazonium salt) or the final azo dye showed no selective staining of mast cells. Enzymatic activity was completely abolished by di*iso*propylfluorophosphate (10^{-4} *M*), copper sulphate (2×10^{-3} *M*) and lead nitrate (2×10^{-3} *M*), but potassium cyanide (5×10^{-4} *M* and 10^{-2} *M*), sodium iodoacetate (10^{-3} *M*) and soybean trypsin inhibitor (1 mgm./ml.) failed to affect enzyme activity significantly. When stable tetrazotized di*ortho*anisidine[8] was used as the diazonium component in the histochemical system, inhibition of enzymatic activity was complete. This diazonium salt did not inhibit hydrolysis of 3-chloroacetyl-2-naphthoic acid anilide by mast cells.

Test-tube experiments with 0·5 ml./mgm. portions of salt-free crystalline α-chymotrypsin and trypsin (bovine) using N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride incorporated as substrate at a concentration of 5 mgm./ml. confirmed the failure of hydrolysis of N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride by chymotrypsin in 18 hr., and substantiated the rapid hydrolysis of the substrate by trypsin[3]. The method used for demonstration of hydrolysis in test-tube experiments was similar to that previously described[3] in which N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride alone was incubated in the presence of enzyme and buffer. The released naphthylamine was estimated, following removal during incubation of aliquot samples, by azo coupling and extraction of the azo dye product. In a separate experiment the same stabilized diazonium salts used in the original histochemical procedure[6,7] were incorporated (0·5 mgm./ml.) in the trypsin incubating medium. No hydrolysis by trypsin as determined by extraction of azo dye was noted. This indicated that inhibition of trypsin activity by the diazonium salt or its stabilizer had occurred, though this inhibition did not occur in the histochemical system. Preliminary experiments to demonstrate competitive inhibition by the method of Benditt and Arase[9] revealed inhibition by N$^{\alpha}$-benzoyl-*l*-arginine amide hydrochloride, a substrate rapidly hydrolysed by trypsin but not by chymotrypsin. Tissues of rat, rabbit, guinea pig, mouse, dog and

human were examined for evidence of hydrolysis of N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride by mast cells and only the latter two revealed enzymatic activity.

It is evident from the above results that the species-specific enzyme in mast cells demonstrated using N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride as substrate has properties that relate it to, but do not identify it with, trypsin. The mast cell enzyme requires no apparent activation, is irreversibly inhibited by di*iso*propylfluorophosphate and competitively inhibited by N$^{\alpha}$-benzoyl-*l*-arginine amide hydrochloride, but is not inhibited by soybean trypsin inhibitor or specific diazonium salts[6,7]. It differs from the enzyme described by Benditt in its apparent absence from rodent mast cells and its inhibition by a specific diazonium salt[8]. The competitive inhibition of N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride by N$^{\alpha}$-benzoyl-*l*-arginine amide hydrochloride is indicative, but not definitive evidence of its dissimilarity from the chymotrypsin-like enzyme. The fact that N$^{\alpha}$-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride is not a substrate for α-chymotrypsin further substantiates this distinction. Inhibition of the trypsin-like enzyme by di*iso*propylfluorophosphate and lack of inhibition by cyanide distinguishes it from the aminopeptidase-like enzyme described in mast cells[10].

The physiological function of this trypsin-like enzyme in mast cells of certain species is not known, but it may possibly act to liberate secretory granule substance. It has been shown that lysis of mast cell granules is, in fact, proteolysis[11] and that activation of a trypsin-like enzyme (present in an inactive state in guinea pig mast cells) produces in these cells a hydrolysis of protein and release of histamine[12]. It has further been demonstrated that heparin is bound to protein at the time of its release from mast cells and that its release is dependent upon proteolysis[13]. It is therefore conceivable that as the result of a variety of injurious stimuli, a trypsin-like enzyme is either activated (rodents) or released from *in situ* inhibition (humans and dogs) within the mast cell cytoplasm. The incident intracytoplasmic proteolytic action of this enzyme may then liberate secretory granule substance[14].

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[1] Benditt, E., and Arase, M., *J. Exp. Med.*, **110**, 451 (1959).
[2] Riedel, A., and Wünsch, E., *Hoppe-Seylers Z. physiol. Chem.*, **316**, 61 (1959).
[3] Holland, G. F., and Cohen, L. A., *J. Amer. Chem. Soc.*, **80**, 3765 (1958).
[4] After this synthesis had been completed, preparation of Nα-benzoyl-*dl*-arginine-β-naphthylamide hydrochloride via the carbodiimide procedure was reported (cf. ref. 2).
[5] Gomori, G., *J. Histochem. Cytochem.*, **1**, 469 (1953).
[6] Fast corinth V from General Dyestuffs Co., New York.
[7] Fast garnet *GBC* from General Dyestuffs Co., New York.
[8] Fast blue *B* from General Dyestuffs Co., New York. Michrome blue 603 (diazotized 4′-amino-2′,5′-dimethoxybenzanilide), from Edward Gurr, Ltd., London, may also be used.
[9] Benditt, E., and Arase, M., *J. Histochem. Cytochem.*, **6**, 431 (1958).
[10] Braun-Falco, O., *Nature*, **183**, 51 (1959).
[11] Hill, M., *Nature*, **180**, 654 (1957).
[12] Ungar, G., and Damgaard, E., *J. Exp. Med.*, **101**, 1 (1955).
[13] Snellman, O., Sylvén, B., and Julén, C., *Biochim. Biophys. Acta*, **7**, 98 (1951).
[14] Keller, R., *Int. Arch. Allergy*, **11**, 328 (1957).

ANIMAL PHYSIOLOGY

Excretion of Rose Bengal in Bile

The metabolism of rose bengal has been investigated by us in humans as well as in experimental conditions in animals. When we examined the dye by one-dimensional paper chromatography using 25 per cent ethanol and 5 per cent ammonia (1 : 1) as a solvent[1], we found it contained several components.

When a commercial preparation of rose bengal (Hopkins and Williams) was examined in ethanol–ammonia at least six components were distinguished in visible light, five red or orange-red (*R*1–*R*6) and one yellow (*Y*). The biggest spot was a red component (*R*3) with an R_F-value of about 0·50. Two other red components with R_F-values of 0·62 (*R*4) and 0·70 (*R*5) were second and third, respectively, in size. The other components appeared less prominent. In ultra-violet light all the above-mentioned components were seen fluorescing, the yellow spot having the brightest fluorescence. At least four faintly fluorescing spots (*F*1–*F*4), which could not be detected in visible light, were seen in ultra-violet light. In addition, radioactive preparations of rose bengal (Abbott) labelled with iodine-131 have been examined, and the distribution of radioactivity on the chromatogram estimated by scintillation counting and autoradiography. Some 51 per cent of the radioactivity was found localized to the spot *R*3. The *R*4 and *R*5 components contained 38 and 9 per cent respectively of the total radioactivity. We have found that there are great variations of the distribution of the radioactivity in the components *R*3–*R*5 of different batches of radioactive rose bengal, but the sum of radioactivity of *R*3–*R*5 usually amounts to more than 90 per cent of the total radioactivity. A slight blackening corresponding to the yellow spot was seen on the autoradiogram. Other details are seen in Fig. 1.

After intravenous injection into rats of the radioactive rose bengal together with a suitable amount of carrier, bile was collected and the excreted dye was extracted in ether after acidification of the bile. The ether-extracted rose bengal was subjected to paper chromatography in ethanol–ammonia. All components of the injected dye were recovered and

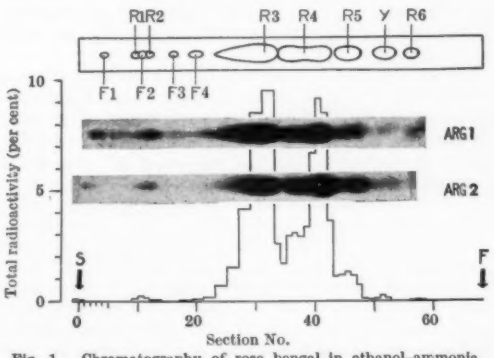

Fig. 1. Chromatography of rose bengal in ethanol–ammonia. *S*, starting point; *F*, solvent front. *R*1–*R*6, red components, and *Y*, yellow component, seen in visible light; *F*1–*F*4, fluorescing components only seen in ultra-violet light of the stable dye. *ARG* 1, autoradiogram of a chromatogram of a radioactive stock solution of rose bengal. *ARG* 2, autoradiogram of a chromatogram of radioactive rose bengal recovered from bile. The diagram shows the distribution of radioactivity on the chromatogram

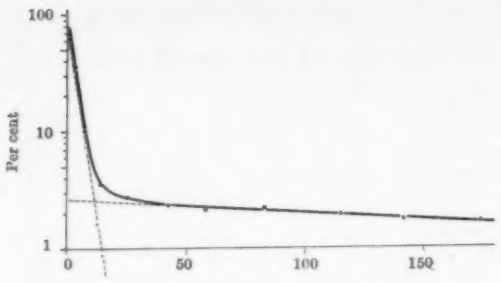

Fig. 2. Blood disappearance curve of radioactive rose bengal in the rat

in about the same proportions. Even the yellow component was excreted in the bile (Fig. 1).

The blood disappearance curve of rose bengal in the rat after injection of an iodine-131 labelled dye could be expressed as an approximate bi-exponential function. The disappearance rate of one phase was very slow; that of the other rather fast. When extrapolated to zero, the slow phase did not amount to more than 4 per cent of the dose injected (Fig. 2).

In accordance with Lima and Pieroni[2], we have found that rose bengal is not a pure compound but a mixture of several chemical substances. The explanation of the bi-exponential blood disappearance curve, however, can scarcely be based on this fact because the liver, as indicated above, is capable of removing all components of rose bengal from the blood. Furthermore, since the proportions between the components are roughly the same in the bile as in the injected material, no other organ seems to be responsible for a specific removal of any of the components. There is still a possibility that the liver might remove one of the small components of rose bengal from the blood at the slower rate. Thus we injected large amounts of radioactive rose bengal into rats. When the blood disappearance curve had reached its 'tail' portion the animals were killed and blood serum collected. When this radioactive serum was injected into rats the usual bi-exponential blood disappearance curve of rose bengal appeared.

The bi-exponential curve can be explained by assuming that there is a back-flow into the blood of a fraction of the amount of rose bengal in the liver cells. It then appears as if the percentage of the dye eliminated from blood will diminish in time and reach a low value.

We have observed that a back-flow to the blood of the rose bengal of the liver cells really can occur; for example, when rose bengal is injected 15 min. after bile obstruction the usual initial fall in the blood curve is followed by an increase in the blood radioactivity.

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[1] Ishida, Y., Inagaki, N., Shiota, A., and Watanabe, R., *J. Pharm. Soc. Japan*, **73**, 736 (1953).
[2] Lima, F. W., and Pieroni, R. R., *Nature*, **184**, 1065 (1959).

Inhibitory Actions of γ-Aminobutyrylcholine

Since the existence of γ-aminobutyrylcholine in the mammalian brain and the possibility of its being a neurotransmitter have been reported[1], new findings on this substance have been reported by several investigators. Takahashi *et al.*[2] noticed its inhibitory effect on the electrical activity of the brain. McLennan[3] found that the second active ingredient of his factor 1 can be accounted for by this compound biologically but not chromatographically; and Kewitz[4] claims to have isolated γ-aminobutyrylcholine in crystalline form from the brain of the pig. In view of its anticholinergic and antihistaminic activities[1], we thought it worth while to study further its inhibitory properties on some other preparations.

The isolated heart of a clam, *Meretrix lusoria* Röding[4], reacted to γ-aminobutyrylcholine with decrease of tonus and the amplitude of beats. This action could be observed at a concentration of 10^{-2} *M* but not at 10^{-3} *M*. Washing removed all the effect. Similar action was exhibited by another choline ester of amino-acid like glycylcholine which was even more potent than γ-aminobutyrylcholine in this respect, and was active at the concentration of 10^{-4} *M* but not at lower concentrations. Unlike γ-aminobutyrylcholine, γ-aminobutyric acid caused short lasting tonic contraction of the heart at 10^{-1} *M*, but it was inactive at 10^{-2} *M*.

In the crayfish intestine, inhibitory potency of γ-aminobutyric acid and of γ-aminobutyrylcholine was about the same, both being effective up to 10^{-4} *M* but not effective at 10^{-5} *M*.

On the neuromuscular preparation of the crayfish claw[5], γ-aminobutyric acid and γ-aminobutyrylcholine elicited qualitatively similar action, inhibiting contraction of the indirectly stimulated closer muscle. After injection of either one, the height of its contraction showed a continuous decrease, but their effect was removed by washing with pure crayfish solution. γ-Aminobutyric acid was more potent; at 10^{-1}–10^{-3} *M* it caused complete block of the contraction and at 10^{-4} *M* the inhibitory action was still distinctly noticeable. γ-Aminobutyrylcholine also caused complete loss of contractility at 10^{-3} *M*, but not at lower concentrations, though its inhibitory effect was still noted at 10^{-4} *M*. Butyrylcholine was less potent than γ-aminobutyrylcholine in this inhibitory action, and glycylcholine was much less active.

Thus, there are some differences in potency and quality of their inhibitory actions, depending upon preparations used for study.

The most pronounced effect was observed on the cat's sciatic-gastrocnemius preparation of Brown. Following intra-arterial injection of 0·02 μgm. of γ-aminobutyrylcholine, the muscular contraction by indirect stimulation decreased in height rapidly, then disappeared, but resumed later (Fig. 1). With higher dose (20·0 μgm.), increase of the tonus set in. With

Fig. 1. Kymograph record of the contraction of cat's gastrocnemius muscle stimulated through the sciatic nerve with square wave stimulator. γ-Aminobutyrylcholine, 0·02 μgm. At the point indicated, it was injected intra-arterially in the dose of 0·02 μgm.

much higher dose (200·0 μgm.), the muscle reacted with irregular fibrillary twitches, instead of normal contraction, to the stimulation of the nerve, and after a while it became quite unresponsive. Such an extreme potency of γ-aminobutyrylcholine on the mammalian neuromuscular transmission is noteworthy. Besides, this property is dissimilar from that of the inhibitory factor of McLennan, which is reported not to affect this preparation[6]. Glycyl-, propionyl-, and butyryl-choline were also inactive at doses of 0·4, 4·0, and 40·0 μgm., except that 40·0 μgm. of propionylcholine caused temporary fall of the size of contraction.

Thus, γ-aminobutyrylcholine exerts a definite inhibition on various activities. A part of it may be due to its anticholinergic activity already reported[1]. However, since it has other properties such as antihistaminic action in addition, its inhibitory activity should not be ascribed solely to competition at the acetylcholine receptor. Further studies are necessary for understanding the mechanism of its inhibitory actions.

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[1] Kuriaki, K., Yakushiji, T., Noro, T., Shimizu, T., and Saji, Sh., *Nature*, **181**, 1336 (1958).
[2] Takahashi, H., Nagashima, A., and Koshino, Ch., *Nature*, **182**, 1443 (1958).
[3] McLennan, H., *J. Physiol.*, **146**, 258 (1959).
[4] Kewitz, H., *Naturwiss.*, **46**, 495 (1959).
[5] Yoshihara, H., and Kuriaki, K., *C.R. Soc. Biol.*, **151**, 1462 (1957).
[6] Florey, E., *Canad. J. Biochem. and Physiol.*, **34**, 669 (1956).
[7] Florey, E., and McLennan, H., *J. Physiol.*, **129**, 384 (1955).

Fracture Healing by Vitamin K

The aim of this short communication is to report on the pronounced effect of vitamin K in speeding up the rate of bone healing. Some clinical observations (Bouckaert, A., and Hart Kliniek, H., personal communication) and preliminary experiments (Bouckaert, J. H., and Oyaert, W., personal communication) indicated that vitamin K had a real effect on all collagen tissues and especially bone tissue.

Adult male and female rabbits and rats were used in this study. Complete transverse fractures of the left tibia were produced by an open operation under ether anæsthesia. In the case of rabbits intramedullary pins were used to fix the fractured ends of the bone. In rats the fractures were supported by maintaining the fibulæ intact. The operation wound, which was thereafter closed with catgut, healed uneventfully in nearly all cases. All animals were kept under the same dietary conditions on a well-balanced ration, fed and watered *ad libitum*. Various groups of animals received vitamin K while others served as controls.

Fig. 1*a* is a radiomicrograph of a fractured rabbit tibia after treatment with vitamin K. It shows a well-developed periosteal callus and the interfragmentary space woven with primary bone in a comparatively high degree of mineralization. Fig. 1*b* is a radiomicrograph of a control animal which received no medicaments. Here the periosteal callus is not

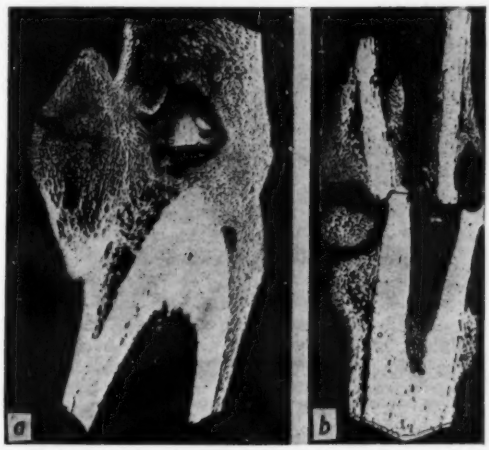

Fig. 1. Radiomicrographs from fractured tibiæ of *a*, an animal receiving vitamin K; *b*, a control animal

complete. The space between the fractured ends is occupied with tissues devoid of any mineral deposition. Ordinary radiographs showed a complete continuity of the fractured bone of the treated animal in contrast to persistence of the fracture site in the control.

Full details about the further steps in our study with vitamin K singly or combined with other therapeutics as well as its exact mode of action will appear elsewhere.

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Effect of Fœtal Hypophysectomy on the Fœtal Liver Fat in the Rabbit

Angevine[1], in his study of the pituitary and adrenals of 20 human anencephalic fœtuses, noted that in practically every case the subcutaneous pannus of fat was unusually thick especially over the shoulders and thorax. This observation has been confirmed in a number of full-term or near full-term anencephalics which are now being studied. Jost[2] has recently shown that fœtal thyroidectomy in the rabbit results in an increase in total fat (determined by ether–alcohol extraction), which does not occur if thyroxine is injected into the fœtus at the time of the thyroidectomy. This raises the possibility that the increase in fat in the human abnormality is due to a fœtal hormonal disturbance, and this view is supported by the knowledge that the pituitary of anencephalics is reduced in size and that secondary to this the adrenals are extremely small[1]. It is therefore possible that the hypofunction of the pituitary either directly, or indirectly by affecting other endocrine glands, determines the increase in fat found in the human anencephalic monster.

To study this aspect of anencephaly further it was decided to investigate the effect of removing the

A B C D

Fig. 1. Effect of fœtal decapitation in the rabbit. *A*, Control fœtus, 28 days old; *B* and *C*, fœtuses decapitated at 21 days and developed *in utero* to 28 days; *D*, a 21-day rabbit fœtus, showing the size of the fœtus at the time of the operation

fœtal pituitary in the rabbit on the total fat content of the fœtal liver.

Five rabbit fœtuses were decapitated (using the method described by Jost[3]) to remove the fœtal pituitary gland. The operation was performed between the twentieth and twenty-first day after mating, and the headless fœtuses were left within the uterus to continue growing. After a further seven to eight days of intrauterine development the fœtuses and the litter mates were removed by Cæsarian section, giving a total of five decapitated fœtuses and fifteen litter mate controls for study. The weights of the operated fœtuses were determined and compared with the headless weights of the control fœtuses.

The liver was dissected from all fœtuses and the fats extracted with ether and expressed as a percentage of the dry weight of the liver.

It was found that although the decapitated fœtuses were very slightly smaller by weight than the headless controls, this difference was not significant. Fig. 1 shows two 28-day fœtuses which were decapitated at 21 days, compared with a 28-day litter mate control; the small fœtus is 21 days old and shows the size at the time of decapitation.

Table 1 shows the results of the estimation of liver fat.

Table 1. Total Fat of Liver of Decapitated and Control Rabbit Fœtuses

Fœtuses	Age at death	No.	Total liver fat (per cent of dry weight)	± *S.E.*
Controls	28–29 days	15	41·3*	± 1·5
Decapitated (at 20–21 days)	28–29 days	5	49·4*	± 2·3

* This difference is significant. $P < 0.01$.

These results demonstrate that the operated rabbit fœtus had a significantly greater content of fat in the liver than the controls. This effect is likely to be due to the removal of the fœtal pituitary, and the work of Jost[3] suggests that the effect may be mediated secondarily through the fœtal thyroid.

These findings, together with those of Jost[3], suggest that the increase in fat noted by Angevine[1] in the human anencephalic fœtus and confirmed in this investigation is an endocrine effect resulting from the hypofunction of the fœtal pituitary and possibly other endocrine glands.

It is of interest in this connexion to note that a recent investigation[4] showed that the thyroids of two anencephalics showed the ability to concentrate iodine and make thyroxine.

I am indebted to Profs. E. W. Walls and A. Jost for help and advice in this investigation.

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[1] Angevine, D. M., *Arch. Path.*, **26**, 507 (1938).
[2] Jost, A., and Picon, L., *C.R. Acad. Sci., Paris*, **246**, 1281 (1958).
[3] Jost, A., *Arch. Anat. Micro. Morphol. Exp.*, **40**, 247 (1951).
[4] Yamazaki, E., Noguchi, A., and Slingerland, D. W., *J. Clin. Endocrinol. and Metabol.*, **19**, 1437 (1959).

A Neurohypophyseal Principle in the Elasmobranch Pituitary

The elasmobranch pituitary gland consists of three distinct and largely separate regions (Fig. 1). Two of these, the rostral and ventral lobes, appear to represent the pars anterior of higher vertebrates, while the third, the so-called neurointermediate lobe, consists of intimately interdigitated neural and intermediate tissue[1]. Scharrer[2] has described a pre-optico-hypophyseal system in *Scyliorhinus stellare*, and our own unpublished histological studies on this system in *S. caniculus* have demonstrated the presence of material which is Gomori-positive, aldehyde–fuchsin-positive and stains by the performic acid–alcian blue technique of Adams and Sloper[3]. However, in spite of the undoubted existence of a neurohypophyseal system in elasmobranchs, evidence for the existence of the oxytocic and vasopressor principles characteristically present in the mammalian neurohypophysis is conflicting[4–8]. The investigation reported here attempts to resolve the discrepancy between the morphological and physiological findings.

Pituitary glands were removed from freshly killed *Squalus acanthias*, *Scyliorhinus caniculus*, *Raia clavata*, *R. batis* and *R. naevus*, separated into their constituent lobes, and dried in acetone. The resulting powders were extracted with 0·25 per cent acetic acid by the standard method for ox posterior lobe pituitary, and assayed against international standard posterior lobe pituitary powder, 'Pitocin', or 'Pitressin' (Parke Davis), for oxytocic activity, by the rat uterus and milk ejection methods of Holton[9] and Cross and Van Dyke[10] respectively, for antidiuretic activity[11], and for vasopressor activity[12]. By these means oxytocic, milk ejection and antidiuretic activities were found

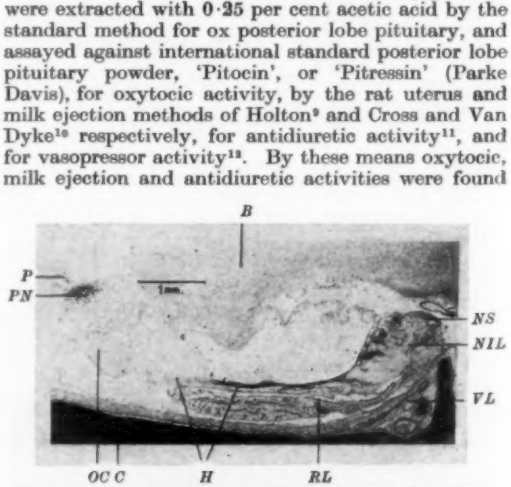

Fig. 1. *Scyliorhinus caniculus*. Parasagittal section of infundibular region of the brain. *B*, Brain tissue; *C*, cartilaginous floor of the skull; *H*, pre-optico-hypophyseal tract; *NIL*, neurointermediate lobe pituitary; *NS*, neurosecretory material in neurohypophyseal region of *NIL*; *OC*, optic chiasma; *P*, parapysis; *PN*, preoptic nucleus; *RL*, rostral lobe pituitary; *VL*, ventral lobe pituitary

Table 1. Oxytocic and Antidiuretic Activities of Elasmobranch Neurointermediate Lobe expressed as m.u./mgm. Acetone-dried Powder

Species	Oxytocic activity (isolated rat uterus)	Oxytocic activity (rabbit milk ejection)	Antidiuretic activity (conscious rat)	Milk ejection oxytocic activity expressed as a percentage of rat uterus oxytocic activity	Antidiuretic activity expressed as a percentage of oxytocic activity	
					Rat uterus	Milk ejection
Squalus acanthias (pregnant ♀♀)	17·0	45	1·3	265	7·6	2·8
Scyliorhinus caniculus	13·2 ± 1·0	60	0·5	450	3·8	0·8
Raia clavata	17·0	60	0·13	353	0·7	0·2
R. batis	7·7 ± 0·5	35	0·6	454	7·7	1·7
R. naevus	10·0	—	0·5	—	5·0	—

to be present and were preferentially distributed in the neurointermediate lobe. No pressor effects were obtained from the amounts of material injected. Table 1 gives a brief summary of some representative results drawn from an extensive series of assays. It is evident from this that :

(1) The potencies, expressed in m.u./mgm. of dried powder, are low compared with international standard posterior lobe pituitary powder. These low figures are largely accounted for by the fact that elasmobranch neurointermediate lobe is composed mainly of intermedia tissue (Fig. 1).

(2) Very little antidiuretic activity is present in any of the elasmobranch powders. This confirms the earlier pioneer work of Heller[7] on dogfish and skate. Values are always below 9 per cent (average 5·1 per cent) of the rat uterus oxytocic figures, and below 2·8 per cent (average 1·2 per cent) of the corresponding milk ejection potency.

(3) The oxytocic activity of neurointermediate lobe powder is always approximately three times as high when measured by the rabbit milk ejection method as when measured by the rat uterus method.

The pharmacological properties of elasmobranch neurohypophyseal extracts have been studied in detail and have been shown to correspond closely to those of their mammalian counterparts. For example : All activities are stable in an acetone-dried powder, and as extracts in 0·25 per cent acetic acid ; the oxytocic activity is unaffected by crystalline pepsin, crystalline trypsin, or atropine ; the oxytocic and antidiuretic activities are destroyed by 0·01 *M* sodium thioglycollate at room temperature, by warming with 0·1 *N* sodium hydroxide, and by ultra-violet light ; the oxytocic activity is destroyed by crystalline chymotrypsin. In 'four-point' rat uterus assays there is no significant deviation from parallelism between the assessed dose response curves for the elasmobranch agent and 'Pitocin'. In contrast to acetylcholine, and extracts of elasmobranch indifferent brain and hypothalamus, and in common with beef oxytocin, the elasmobranch neurointermediate lobe extract shows a long latency of action on the isolated rat uterus.

By definition, the ratios between milk ejection activity, rat uterus oxytocic activity and antidiuretic activity of international standard posterior lobe pituitary powder are 1 : 1 : 1. Analysis of all our results shows that these ratios in the elasmobranch material approximate to 3·5 : 1 : 0·05. A survey of the relevant literature demonstrates that this low titre of antidiuretic hormone relative to oxytocin is unique among the vertebrates so far examined. It seems unlikely that these discrepant ratios in elasmobranchs are due to contaminating inhibitors or activators since both 'Pitressin' and 'Pitocin', when added to elasmobranch neurointermediate lobe extracts inactivated by ultra-violet light, assay at their normal value, and there is the same high degree of destruction of all the activities by the various treatments described above. Recent work on oxytocin analogues has shown that single amino-acid substitutions alter the biological properties of the molecule, and analogues have already been synthesized which are more potent in releasing milk than in causing the rat uterus to contract when assayed against posterior lobe pituitary powder[13].

The antidiuretic activity of the elasmobranch materials examined is approximately 5·0 and 1·2 per cent of the corresponding values for oxytocic activity obtained by rat uterus and milk ejection assays respectively ; these percentages approach the intrinsic antidiuretic activity of synthetic oxytocin. In the light of the low antidiuretic potency of the elasmobranch extracts and the discrepant oxytocic activity ratios, it is suggested that the neurointermediate lobe of the elasmobranch pituitary, so far as it is possible to generalize from five species, is exceptional in containing no discrete vasopressor/antidiuretic principle, though an oxytocic substance, not identical with beef oxytocin, is undoubtedly present. Recent work of Pickering and Heller[14], and Sawyer, Munsick and van Dyke[15], has demonstrated the presence of two oxytocin-like substances in the neurohypophyses of a variety of lower vertebrates (not elasmobranchs). Our range of assays is insufficient to say whether or not a similar situation obtains also in elasmobranchs, though if this is afterwards shown to be the case, it does not affect our submission that a modified type of oxytocin occurs in the elasmobranch neurohypophysis and that the vasopressor/antidiuretic hormone is absent.

This work, a detailed report of which will be published elsewhere, was supported by a generous grant from the Nuffield Foundation.

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[1] van de Kamer, J. C., and Verhagen, T. G., *Proc. Acad. Sci. Amst.*, C, **57**, 358 (1954).
[2] Scharrer, E., *Z. Zellforsch.*, **37**, 196 (1952).
[3] Adams, C. W. M., and Sloper, J. C., *J. Endocrinol.*, **13**, 221 (1956).
[4] Herring, P. T., *Quart. J. Exp. Physiol.*, **6**, 73 (1913).
[5] Herring, P. T., *Quart. J. Exp. Physiol.*, **8**, 245 (1914).
[6] Hogben, L. T., and de Beer, G. R., *Quart. J. Exp. Physiol.*, **15**, 163 (1925).
[7] Heller, H., *J. Physiol.*, **99**, 246 (1941).
[8] Waring, H., and Landgrebe, F. W., in "The Hormones", **2** (New York, 1950).
[9] Holton, P., *Brit. J. Pharmacol.*, **3**, 328 (1948).
[10] Cross, B. A., and Van Dyke, H. B., *J. Endocrinol.*, **9**, 232 (1953).
[11] Ginsburg, M., and Heller, H., *J. Endocrinol.*, **9**, 267 (1953).
[12] Landgrebe, F. W., Macaulay, M. H. I., and Waring, H., *Proc. Roy. Soc. Edin.*, B, **62**, 202 (1946).
[13] Berde, B., Doepfner, W., and Konzett, H., *Brit. J. Pharmacol.*, **12**, 209 (1957).
[14] Pickering, B. T., and Heller, H., *Nature*, **184**, 1463 (1959).
[15] Sawyer, W. H., Munsick, R. A., and van Dyke, H. B., *Nature*, **184**, 1464 (1959).

HÆMATOLOGY

Paradoxical Effect of Magnesium Ions on Blood Coagulation

We have recently reported on the delay of whole-blood thrombin generation *in vitro* and *in vivo* which occurs when the concentration of magnesium is raised[1,2]. These results might have been thought to contradict the fact that addition of magnesium is known to enhance clotting of blood of which the calcium content has been lowered by previous passage through a suitable ion exchange column[3].

The inhibitory effect of magnesium on untreated blood has been explained on the basis of a competition between magnesium and calcium in processes where calcium is the more efficient catalyst[4]. We want to suggest that the present apparent contradiction can also be explained on similar lines because the outcome of the competition will depend on the concentration of either ion.

In decalcified plasma, magnesium alone will not restore coagulability. However, when sub-optimal amounts of calcium are added, magnesium will then enhance clotting. This can be understood if one assumes that some part or parts of the clotting mechanism can be activated by calcium alone, and that others can utilize magnesium though as a less efficient catalyst. Thus, if the amount of calcium is sub-optimal magnesium will be calcium-sparing; but if enough calcium is available to serve all clotting processes, added magnesium can then only displace the more efficient catalyst, and its effect will therefore be to retard clotting. Hence, the effect of a given concentration of magnesium on clotting will depend on the calcium concentration. Table 1 shows that with a calcium concentration of 5 m.equiv. per l., 10 m.equiv. per l. of magnesium is inhibitory, and with 2 m.equiv. per l. of calcium, 10 m.equiv. per l. of magnesium activates.

Table 1. Clotting Times (min.) of De-ionized Plasma diluted 10 : 1 with Additive

Ca^{++} (m.equiv. per litre)	Mg^{++} (m.equiv. per litre)			
	–	2	4	10
2	>60	11	12	32·5
3	15			
4	8·5			
5	7·5	7	8	11
6	7			
8	7			
10	7			
15	7	7	8	10
20	7·5			
30	9·5			
40	15			
50	>60			

Blood was taken into an ion exchange blood pack (Fenwall Laboratories, U.S.A.). Plasma was prepared by centrifuging at 1,500 r.p.m. for 20 min. 1 ml. of plasma added to 1 ml. additive in 8 × 75 ml. glass tubes incubated in a 37° C. water-bath. Clotting end-point was taken as the time when tubes could first be inverted without spilling (checked at ½-min. intervals).

As one might expect, with increasing quantities of magnesium the activating effect becomes less, and the inhibitory effect more pronounced. 2 m.equiv. per l. of magnesium will restore the clotting time of a calcium-poor plasma (2 m.equiv. calcium per l.) almost to normal, but will not inhibit the clotting of plasma containing normal amounts of calcium. On the other hand, 10 m.equiv. per l. of magnesium shows only little activation of the calcium-poor plasma, but marked inhibition at physiological levels. Furthermore, with the larger amount of magnesium the inhibition is diminished when calcium is present in high concentrations—bordering on those where calcium itself starts to become an inhibitor. This again illustrates the competition which exists between calcium and magnesium for clotting factors.

(This self-inhibitory effect of high calcium concentration is well-known, and has been explained[4] on a mechanical basis. The divalent calcium ion supposed to link two protein molecules will fail to form this bridge when it is in excess because too many of these molecules will have a calcium atom of their own.)

We wish to thank the Governors of St. Bartholomew's Hospital for a grant towards equipment.

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[1] Anstall, H. B., Huntsman, R. G., Lehmann, H., Hayward, G. W., and Weitzman, D., *Lancet*, **i**, 814 (1959).
[2] Huntsman, R. G., Hurn, B. A. L., and Lehmann, H., *J. Clin. Path.*, **13**, 88 (1960).
[3] Soulier, J.-P., Proc. Seventh European Congress of Hæmatology, 1959 (in the press).
[4] Greville, G. D., and Lehmann, H., *J. Physiol.*, **103**, 175 (1943).

Electrophoretic Patterns of Haptoglobins in Apes

The haptoglobin serum groups are Mendelian characteristics of normal people as are blood groups. Two common genes, Hp^1 and Hp^2, have been found and their frequencies in all white peoples studied have been about 0·4 and 0·6 respectively[1–7]. Gene Hp^2 seems to be much rarer in Negroes than in Whites[1,5].

The sera of most mammalian species seem to contain haptoglobin, but the only electrophoretic pattern that we have found in domestic and laboratory animals studied resembles closely that of Hp^1Hp^1 (*Hp* 1–1) people. On account of this observation we have studied blood samples of different primates listed below with the starch gel electrophoresis method described earlier[6].

Macacus rhesus	17 specimens
Macacus radiata	10 ,,
Papio hamadryas	1 ,,
Cercopithecus aethiops	1 ,,
Pan troglotydes	2 ,,

Sera of all apes studied gave the pattern *C* of Fig. 1, which closely resembles the pattern *D* (human *Hp* 1–1).

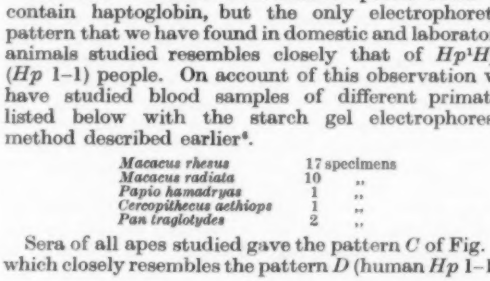

Fig. 1

It thus seems that if there is any haptoglobin polymorphism among *M. rhesus* or *M. radiata* demonstrable by starch gel electrophoresis, the gene giving *Hp* 1–1 like pattern is predominant.

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[1] Sutton, H. E., Neel, J. V., Binson, G., and Zuelzer, W. W., *Nature*, **178**, 1278 (1956).
[2] Laurell, A.-B., and Grubb, R., *Vox Sanguinis*, **2**, 312 (1957).
[3] Galatius-Jensen, F., *Acta Genet.*, **7**, 549 (1957).
[4] Fleischer, E., and Lundevall, J., Proc. Sixth Congr. Europ. Soc. Hæmatol., Pt. 2, 907 (1958).
[5] Allison, A. C., Blomberg, B. S., and Mrs. ap Rees, *Nature*, **180**, 824 (1958).
[6] Mäkelä, O., Eriksson, A. W., and Lehtovaara, R., *Acta Genet.*, **9**, 149 (1959).
[7] Moullec, J., and Fine, J. M., *Nature*, **184**, 196 (1959).

Serum Chromatography on Columns connected to a Common Source of Buffer Gradients

Recently there has been much progress in the separation of serum proteins by column chromatography. The best resolution of the protein components is theoretically obtained by the application of buffer and salt gradients to the column[1]. This principle has been applied during the elution of human serum from modified cellulose anion exchange columns when large numbers of chromatographic peaks were observed[2]. However, this technique is time-consuming, and it is difficult to produce an identical sequence of buffer gradients for each experiment. A modification using a single buffer gradient has been described for the separation of serum components of clinical interest[3], but this method reveals fewer components than the original technique. To overcome these difficulties a method with high resolving power for the simultaneous comparison of sera using the same system of buffer gradients has been developed and investigated.

A chromatographic comparison of human and animal serum on diethylaminoethyl cellulose under the same experimental conditions has not been reported. It was of interest during experiments with bovine serum to compare the results with the behaviour of human serum under identical conditions which were obtained by the simultaneous application to two columns of buffer gradients from the same source.

Experimental details were as follows: two columns (60 cm. × 2·5 cm.) were packed to a height of 25 cm. with diethylaminoethyl cellulose which contained 0·77 m.equiv. of ionizing groups per gm. They were mounted at different positions above an automatic fraction collector in a 4° C. room. The proteins of pooled bovine serum (100 ml.) and pooled human serum (100 ml.) were separated by gradient elution chromatography on separate columns essentially as in the original method[3], but common buffer gradients were generated for both columns in the same 2-l. reservoir connected to a 250-ml. conical flask with magnetic stirrer as mixing chamber. The common solution feed-tube was divided into separate inlet tubes for each column by a Y-shaped glass tube.

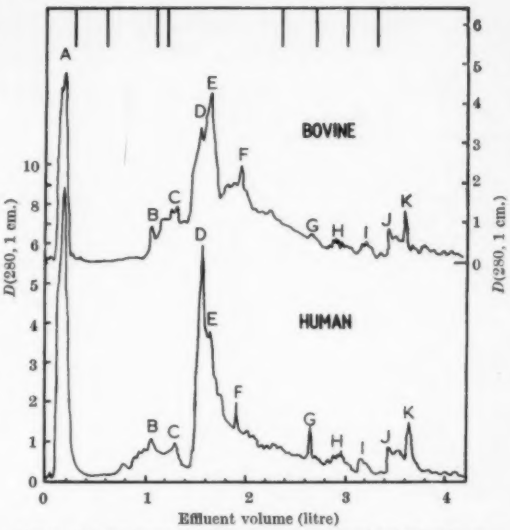

Fig. 1. Elution curves of two sera on diethylaminoethyl cellulose columns connected to the same buffer gradient source. Human serum (lower curve, left-hand ordinate) and bovine serum (upper curve, right-hand ordinate). The ordinates refer to the optical densities of a 1-cm. layer of the column effluent measured at 280 millimicrons wave-length (D(280, 1 cm.)). The positions of buffer changes are shown by the upper vertical lines. On both curves there are peaks emerging at similar effluent volumes. These corresponding peaks are labelled alphabetically in order of their elution

Eluting solutions were forced through this system under regulated air pressure to give a flow through each column of about 20 ml./hr. Solutions used in the buffer reservoir were (*a*) 0·005 *M* sodium phosphate of *p*H 7; (*b*) 0·02 *M* sodium dihydrogen phosphate; (*c*) 0·03 *M* sodium dihydrogen phosphate; (*d*) 0·05 *M* sodium dihydrogen phosphate containing 0·02 *M* sodium chloride; (*e*) 0·05 *M* sodium dihydrogen phosphate containing 0·05 *M* sodium chloride; (*f*) 0·05 *M* sodium dihydrogen phosphate containing 0·1 *M* sodium chloride, and (*g*) 0·05 *M* sodium dihydrogen phosphate containing 0·5 *M* sodium chloride. Effluent fractions of about 10 ml. were collected. The volumes were accurately measured, and the ultra-violet absorption in a 1-cm. cell at 280 mμ was determined for each fraction.

Fig. 1 shows the elution curves of human and bovine serum. During the initial buffer gradient the sharp γ-globulin peak (*A*) emerged. It was followed by a large number of peaks which could be classified into 10 groups (*B*–*K*) each containing chromatographically distinct peaks.

Freeze-dried protein from each fraction was dissolved to give a 2 per cent solution which was examined by paper electrophoresis in 0·075 ionic strength veronal buffer of *p*H 8·6 followed by protein staining with bromphenol blue dye. As previously noted by Sober *et al.*[2], some difficulty was encountered in attempting to classify the results solely on the basis of mobilities. However, the patterns of the different groups showed the following distinct features: (*A*) protein only in the γ position, (*B*) protein in the β and γ regions, (*C*) protein in the γ position, (*D*) and (*E*) protein in the α and albumin regions, (*F*) major protein in the albumin region, (*G*) and (*H*) protein in the α, β and albumin positions, (*I*), (*J*) and (*K*) protein in the α and β regions. In addition, all the fractions showed some protein in the γ-globulin

area of the paper strips. It was noticed that corresponding peaks had similar electrophoretic composition and emerged at the same effluent volumes from both sera.

This suggests the possible identity of the components from both sera and shows the reproducibility obtained on two columns by this method. The differences in the heights of corresponding peaks of the two sera probably reflect the known species differences in the electrophoretic composition of these sera.

The results suggest that this method may be useful for the comparison of different samples of animal sera. These sera are commercially important in the production of antibodies and as tissue-culture nutrients. Large-scale separation of these proteins on a battery of columns connected to the same buffer source may have advantages over single large-column operation, since the homogeneous packing, handling, regeneration, thermal and mechanical stability, and vertical space requirements may be better in the former case. Analytical applications for the comparison of pathological sera with a normal control serum may also be of interest, as modified cellulose columns have recently been described for the separation of myeloma proteins[3], rheumatoid factor[4], and lupus erythematosus factor[5] from human serum.

The present work is part of a project on the chromatography of high molecular weight substances. The help and support of the Director of the Commonwealth Serum Laboratories during this work is gratefully acknowledged.

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[1] Alm, R. S., Williams, R. J. P., and Tiselius, A., *Acta Chem. Scand.*, **6**, 826 (1952).
[2] Sober, H. A., Gutter, F. J., Wyckoff, M. M., and Peterson, E. A., *J. Amer. Chem. Soc.*, **78**, 756 (1956).
[3] Fahey, J. L., McCoy, P. F., and Goulian, M., *J. Clin. Invest.*, **37**, 272 (1958).
[4] Williams, R. R., Stewart, L. C., and Jenkins, J. C., *Proc. Soc. Exp. Biol. Med.*, **99**, 554 (1958).
[5] Willkens, R. F., Dreschler, M., and Larson, D. L., *Proc. Soc. Exp. Biol. Med.*, **99**, 645 (1958).

Decrease of the Copper Bands of *Helix pomatia* Hæmocyanin

A study of the absorption spectra of α- and β-hæmocyanin of *Helix pomatia* indicated that the copper bands of α-hæmocyanin, with their maxima at 346 and 580 mμ, are not quite reproducible[1]. As a continuous decrease of the copper-bands was liable to occur, the extinction of hæmocyanin preparations has been followed over several months.

The blood of several snails was collected and centrifuged during 20 min. at 10,000 r.p.m. in a water-cooled Pirouette (Phywe, Göttingen, Germany). Samples were treated as quickly as possible in various ways and stored at 4° under toluene in well-stoppered flasks. Care was taken to ensure full saturation with toluene during the whole procedure. The extinctions were measured in a Beckman *DU* spectrophotometer at 346 and 278 mμ on aliquots, immediately after dilution with a saturated borax buffer of *p*H 9·18. At this *p*H the contribution of light scattering to the total extinction is practically negligible[2].

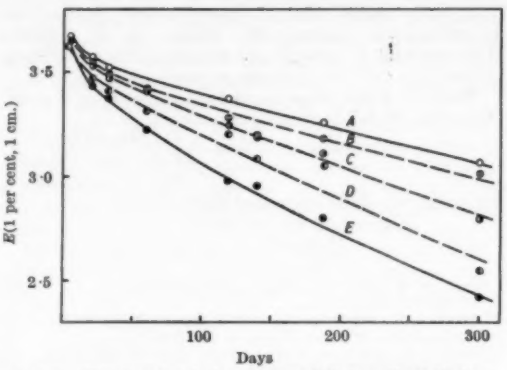

Fig. 1. The decrease during storage of the specific extinction coefficient of *Helix pomatia* hæmocyanin at 346 mμ

The specific extinction coefficients were calculated for the copper band at 346 mμ as a function of E(1 per cent, 1 cm.) = 14·16 at 278 mμ. As Fig. 1 shows, the extinctions gradually fall off, starting from the same value. The decrease is most pronounced for blood precipitated three times with ammonium sulphate (*p*H 5·3). The precipitate was dissolved in 0·1 *M* sodium acetate buffer, *p*H 5·7, after the first and the second precipitation. The third time it was stored with the supernatant solution (*E*). The drop of the extinction is smaller when this triple precipitation is followed by dialysis during one night against water at 4°, the precipitate having been dissolved in 0·1 *M* NaOAc, *p*H 5·7 (*C*). The protection is rather good after filtration of 100 ml. of blood on 10 gm. of activated charcoal ('Norit') (*B*) and very small after extraction with 1 volume of chloroform (*D*). Two ultracentrifugations (Spinco Model *L*, rotor No. 30, 2½ hr., 4°, 78,410*g*), followed by dialysis during 48 hr. against water (*A*), yielded good protection, comparable to the charcoal treatment (*B*).

The general shape of the copper bands remains unchanged, as indicated by a comparison of freshly prepared blood with some samples after 300 days storage. It is to be noted further that the extinction coefficients at 580 mμ follow exactly the corresponding values at 346 mμ (Table 1).

Table 1. Relationship between the Specific Extinction Coefficients of the Copper Bands after 300 Days Storage (Dilution with a Saturated Borax Buffer, *p*H 9·18, 20° C.)

Treatment	E(1 per cent, 1 cm.) 346 mμ	E(1 per cent, 1 cm.) 580 mμ	E_{346}/E_{580}
D	2·55	0·154	16·5
C	2·79	0·171	16·4
B	3·01	0·179	16·8
A	3·06	0·185	16·5

In order to see if α- and β-hæmocyanin behave in a similar way, preparations of total hæmocyanin, made from blood collected during different years and stored under toluene at 4° as an ammonium sulphate precipitate for varying periods, were separated into the two proteins. Extinction coefficients at 346 mμ, determined at *p*H 9·18, are given in Table 2. For β-hæmocyanin four values out of five are very near 2·89. A high value of 3·30 was obtained on a β-preparation, prepared as quickly as possible from fresh blood. The extinction coefficients of some α-hæmocyanin preparations, stored under similar conditions, range from 1·78 to 2·61.

Table 2 Specific Extinction Coefficients of the Copper Band at 346 mμ (Purified Preparations, Diluted with a Saturated Borax Buffer, pH 9·18, 20° C.)

Preparation	Year of collection	Protein concentration (per cent (w/v))	E(1 per cent, 1 cm.)
β-Hæmocyanin	1952	0·1060	2·98
	1956	0·321	2·88
	1957	0·0563	2·90
(very fresh)	1957	0·0399	3·30
	1959	0·281	2·79
α-Hæmocyanin	1952	0·240	1·78
	1956	0·397	2·20
	1956	0·334	2·58
	1957	0·100	2·60
	1957	0·202	2·07
	1958	0·236	2·61
	1959	0·194	2·21
	1959	0·156	2·10

These observations clearly establish the parallel decrease of the copper bands of *Helix pomatia* hæmocyanin under ordinary storage conditions, β-hæmocyanin changing more slowly than α-hæmocyanin. To explain the decrease a bacterial action may well be ruled out, as the effect is highest in the presence of half-saturated ammonium sulphate. An organic substance, possibly aromatic, which is salted out by ammonium sulphate, could be responsible for this spectral decrease. Evidence has indeed been given for the presence of phenolic amines, for example tyramine, in the blood of some cephalopods[3].

Related observations have been made on the oxygen binding of hæmocyanins. A preparation of *Limulus polyphemus*, stored for several months under half-saturated ammonium sulphate, showed a 30 per cent decrease of the absorption band in the visible, which was accompanied by a 26 per cent lowering of the oxygen-binding capacity[4]. The hæmocyanin of *Helix pomatia* has further been reported to show during storage at − 10° a change from the sigmoid oxygenation curve to a rectangular hyperbola[5].

The ratio, however, of α- and β-hæmocyanin was completely normal in the preparations *A* to *E* after 300 days storage, as determined by dissociation tests in a Spinco ultracentrifuge model *E* and *M* sodium chloride at *p*H 5·8.

The antigenicity moreover of some hæmocyanin solutions, kept under toluene at 4°, did not undergo any noticeable change after ten years[6].

In conclusion it may well be stressed that the usual way of storing hæmocyanins as an ammonium sulphate precipitate, without any other treatment, proves to be the least satisfactory, if properties connected with the oxygen-binding sites are to be studied.

We wish to thank the Nationaal Fonds voor Wetenschappelijk Onderzoek for a postdoctorate fellowship to one of us (K. H.) and for several grants towards the expenses of this investigation.

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[1] Heirwegh, K., Borginon, H., and Lontie, R., Fourth Int. Congr. Biochem., Vienna, 2 (1958).
[2] Lontie, R., Dessent, M., and Heirwegh, K., *Meded. vlaamse Acad. Kl. Wet.*, **13**, No. 1 (1951).
[3] Bacq, Z. M., and Ghiretti, F., *Arch. Int. Physiol.*, **59**, 288 (1951).
[4] Redfield, A. C., *Biol. Bull., Woods Hole*, **58**, 238 (1930).
[5] Horsmans, J., after Lontie, R., *Meded. vlaamse chem. Ver.*, **16**, 110 (1954).
[6] Litt, M., and Boyd, W. C., *Nature*, **181**, 1075 (1958).

PLANT PHYSIOLOGY

Vitamin E and Flowering of *Fragaria vesca* L. var. *semperflorens* Duch.

The development of *Fragaria vesca* L. var. *semperflorens* Duch. is very sensitive to day-length[1]. In short-day conditions the plants do not reach the reproductive stage. Days more than 12 hr. long are required. Short-day treatment also inhibits flower formation of *Fragaria vesca* plants which have previously reached the flowering stage in long-day conditions. During a season in the open field, the number of flowers found on such flowering plants varies according to the day-length as shown in Fig. 1. A maximum of flowers is found in the inflorescences growing during August–October, which have been initiated during June–July, in the longest days.

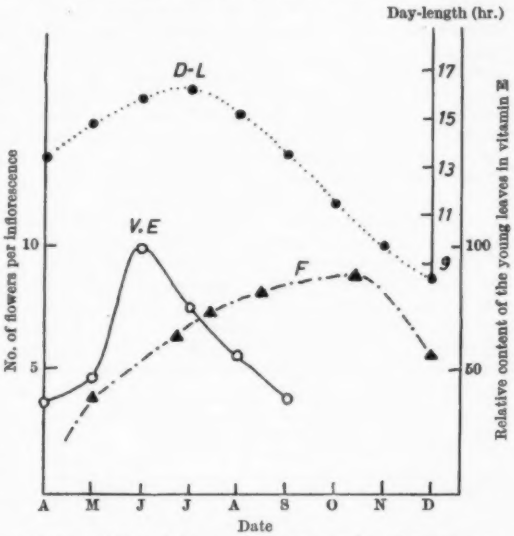

Fig. 1. Relations between day-length, number of flowers per inflorescence and vitamin E content of the young leaves in flowering plants of *Fragaria vesca* L. var. *semperflorens* Duch. (for explanations, see the text; *D-L*, day-length; *V.E.*, vitamin E content; *F*, number of flowers per inflorescence)

On the other hand, in some experimental conditions, runner plants of *Fragaria vesca* have been shown to improve their flowering capacity when vitamin E is applied externally to the leaves in low concentration[1]. It seemed, therefore, interesting to investigate the two following points: (1) Is the vitamin E content of *Fragaria vesca* plants grown in 8-hr. day (remaining vegetative) different from that of plants grown in 16-hr. day (beginning to form flowers)? (2) Is the vitamin E content of flowering plants related in some way to their flowering intensity?

Vitamin E was measured by the method of Meunier and Vinet[2] in the unsaponifiable fraction of the leaves extracted as described by Sironval[1]. The separation of the vitamin was obtained on 'Floridin *XS*' prepared following Kjölhede's method[2].

Table 1, measurements 1–3, answers the first question. The measurements were made in adult leaves of *Fragaria vesca* plants grown in 8-hr. days (remaining vegetative) or in 16-hr. days (beginning to form their first flowers). The temperature was maintained constant (20° C.) for measurement 1. Measure-

Table 1. Effect of Day-Length on the Vitamin E Content of the Adult Leaves of *Fragaria vesca* L. var. *semperflorens* Duch. (per γ in 100 mgm. of the Unsaponifiable Fraction)

Measurement No.	Temperature	8-hr. day	16-hr. day
1	20° C. constant	219	430
2	variable	255	490
3	variable	202	480
4	variable	185*	420

* This series has first been grown in long-days. It was transferred to short-days at the flowering stage. The measurement is done 2 months after the transfer (see text).

ments 2 and 3 were made on plants grown in the open field. It appears from Table 1 that the content of vitamin E per 100 mgm. of the unsaponifiable fraction is regularly about twice as high in long-days than in short-days. Calculated on a dry-weight basis, the recorded difference is still more marked.

Measurement 4 of Table 1 is concerned with the second question. Flowering plants grown in 16-hr. day were transferred to 8-hr. day. They were compared to the control remaining in 16-hr. day. After the transfer to short-day the flowering intensity strongly diminished, in accordance with previous observations[1]. Within two months after the transfer, the vitamin E content of the adult leaves of the transferred plants dropped to about half, while the content of the control remained constant.

Another observation concerned with the second question is recorded in Fig. 1, which gives for flowering plants in the field the normal variations of the vitamin E content of the young leaves near the apex (4 cm. high) during the year 1958. These variations are compared with the variations of the flowering intensity and those of day-length. It is seen that the vitamin E content is first low at the beginning of the spring; it rises rapidly until June, and falls after that. The maximum in June corresponds approximately to the maximum of day-length. It appears just before the maximum rate of flower initiation, at the moment of the rapid increase of the number of flowers per inflorescence. This has been found also during the year 1957 (Table 2). However, as shown in Table 2, the seasonal variations of the content in vitamin E is less evident in adult and old leaves than in young ones. The content drops with the ageing of the leaves, as we see when considering the underlined figures of Table 2, which are concerned approximately with the same set of ageing leaves.

It is concluded that the vitamin E content of *Fragaria vesca* leaves is very sensitive to day-length, and that this feature is related in some way to the action of day-length on flower formation. Normal flower formation seems to require a high vitamin E content in the leaves. This is seen not only in experiments with extreme day-length (8-hr. and 16-hr.), but also in ordinary culture in the field. In this last case, the occurrence of a high content in vitamin E in the young leaves surrounding the apex, at the moment of the active increase of flower initiation in June, strongly supports the idea that the vitamin plays a part in the promotion of flowering.

Table 2. Variations of the Vitamin E Content of the Leaves of Flowering *Fragaria vesca* L. cultivated in the Field during the Years 1957 and 1958
(100 for 1957, 62γ per 100 mgm. dry weight; for 1958, 71γ per 100 mgm. dry weight)

Time of observation	Young leaves		Adult, just expanded leaves		Old leaves	
	1957	1958	1957	1958	1957	1958
April	39	37	—	—	—	—
May	28	45	21	27	30	10
June	100	100	25	44	10	38
July	26	73	42	53	12	32
August	18	56	16	38	21	45
September	—	38	26	23	14	11

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[1] Sironval, C., *C.R. Rech. IRSIA, Bruxelles*, **18**, 1 (1957).
[2] Meunier, E., and Vinet, A., *C.R. Acad. Sci., Paris*, **211**, 611 (1940).
[3] Kjölhede, K., *Z. Vitaminforsch.*, **12**, 138 (1942).

Chlorogenic Acid of Lettuce Seeds

The high concentration of chlorogenic acid in lettuce seed has not previously been reported. Fig. 1 compares the ultra-violet absorption spectrum of a methanol solution of chlorogenic acid, 0·02 gm./l., with that of a methanol extract of 1 gm. of lettuce seed made up to 1 litre. The lettuce seed had been extracted continuously with methanol for 16 hr. in a Soxhlet apparatus. Paper chromatography of the extract showed the predominant fluorescent component to be chlorogenic acid. Several other fluorescent components which after elution show the same ultra-violet absorption spectra as chlorogenic acid are also present on the chromatograms. These are probably isomers of chlorogenic acid, but could be other depsides of caffeic acid. Caffeic acid itself is not a major component. Assuming that the compounds which have the same absorption spectrum as chlorogenic acid are isomers and have the same molecular extinction coefficient at 330 mμ, 2 per cent of the weight of the dry seed is chlorogenic acid and its isomers.

In order to investigate the possible role of this large amount of chlorogenic acid, lettuce seeds were germinated for as long as two weeks and the seedlings from 1 gm. of seeds were extracted with methanol.

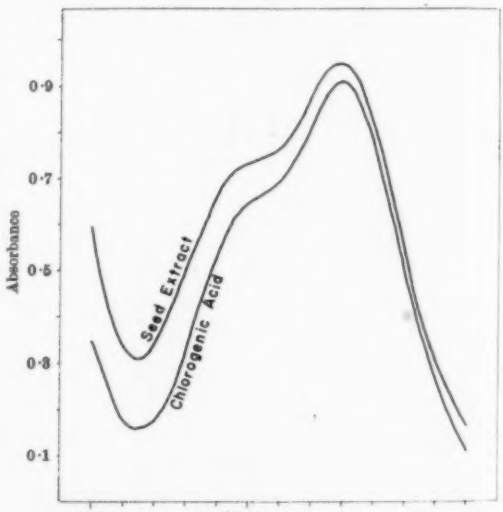

Fig. 1

The total amount of chlorogenic acid did not change during germination. By separating the cotyledons from the seedlings it was shown that the chlorogenic acid remained in the cotyledons of the seedlings with very little, if any, being translocated into the stem or roots. The possibility that chlorogenic acid was actively turning over cannot be ruled out. However, the apparent lack of utilization suggests that chlorogenic acid in lettuce seed may be an end-product which accumulates to a high concentration in the seed, but serves no metabolic function.

The lettuce seed also contains an active polyphenol oxidase as Mayer[1] has shown. Thus, the chlorogenic acid exists in the seed along with a polyphenol oxidase without interacting even in the wet, imbibed seed where metabolic processes are occurring. Mason[2] has suggested theoretically that polyphenol oxidases act as hydroxylating enzymes *in vivo* and assume their oxidase activity only when the cell is broken. According to this view the polyphenol oxidase which destroys the chlorogenic acid when the cell is broken might be involved in its formation and accumulation in the seed.

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[1] Mayer, A. M., *Enzymologia*, **16**, 277 (1954).
[2] Mason, H. S., *Nature*, **177**, 79 (1956).

Nitrogen Feeding of Apple Trees by Post-Harvest Urea Sprays

IN the early post-bloom period it is common to spray apple trees with a 0·5 per cent urea solution which, when repeated several times, results in a clear increase of leaf chlorophyll and leaf total nitrogen in comparison with leaves of unsprayed trees[1]. Higher concentrations may cause leaf injury. It seems unlikely that this injury caused in the late autumn would reduce to any extent the total amount photosynthesized during the passing season. This would permit the use of comparatively concentrated spray solutions at that time. The absorption of nitrogen may be proportional to the concentration of the urea solution[2].

Prior to abscission some 40–50 per cent of the total nitrogen of the leaves is re-absorbed by the tree[3]. This nitrogen makes up the whole, or a part, of the nitrogenous reserves which are utilized the following growing season. Apart from securing the normal nitrogen needs of the tree, a large reserve may effect early starting of growth in the spring in comparison with trees of low reserves[4].

As is shown in Fig. 1, a spray in the middle of October with a 4 per cent urea solution has increased the total organic nitrogen content of the leaves by 51 per cent within two days after application. When attention was first directed to urea sprays as a means of supplying apple trees with nitrogen, the registered increase in leaf nitrogen was approximately 15 per cent after four applications[5]. Fig. 1 shows further that absorbed nitrogen has migrated from the leaves into the tree. By the time of leaf fall (November 12) the sprayed trees had spurs, with developed blossom buds, containing 31 per cent more nitrogen than spurs of unsprayed trees. The corresponding figure for terminal shoots is 16 per cent.

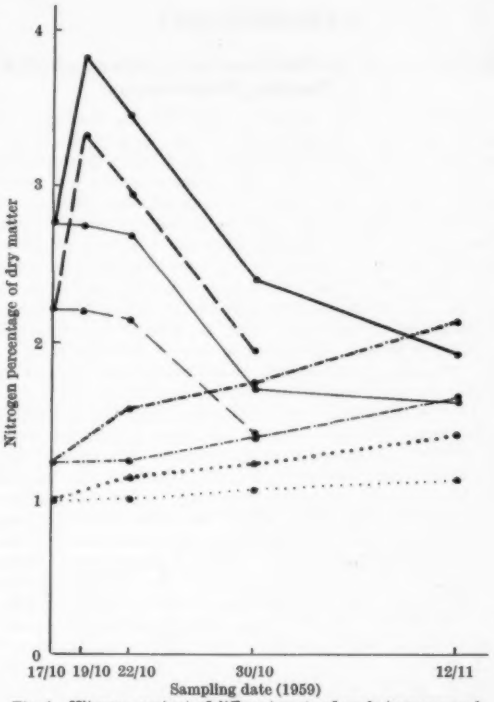

Fig. 1. Nitrogen content of different parts of apple trees sprayed with a 4 per cent urea solution on October 17 (heavy lines), and unsprayed control trees (thin lines). Shoot leaves, ———; spur leaves, — — —; shoots, ...; and spurs, -·-·-·-. All plant parts were washed thoroughly before drying and analysis

The leaf injury caused by the 4 per cent urea spray was some marginal browning of spur leaves and basal shoot leaves, while leaves at the tips of the shoots appeared unaltered.

It is suggested that the amount of nitrogen absorbed by the trees from a single spray might compare to a net intake of 30–40 kgm. nitrogen per hectare. (The amount of leaves in an established orchard is assumed to be of the order of magnitude of 3,000 kgm. dry weight per hectare.) This amount of nitrogen is well above what the trees may be expected to make profitable use of, besides the nitrogen supplied by a normal organic matter metabolism of the soil.

We lack knowledge concerning the responses of apple trees to nitrogen fertilization[6], which is at least partly due to the sources of errors in the experiments carried out. The experience gained during the past autumn, in experiments with the suggested method of nitrogen feeding, seems to promise that accurately known amounts of nitrogen may be introduced into the trees by post-harvest urea sprays. As large effects can be obtained, it would serve profitably in studies on the importance of nitrogenous reserves, as well as in studies on nitrogen metabolism.

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[1] Boynton, D., "Ann. Rev. Plant Physiol.", **5**, 31 (1954).
[2] Boynton, D., *et al.*, *Proc. Amer. Soc. Hort. Sci.*, **62**, 135 (1953).
[3] Murneek, A. E., *Proc. Amer. Soc. Hort. Sci.*, **27**, 228 (1930).
[4] Oland, K., *Physiol. Plant.*, **12**, 594 (1959).
[5] Hamilton, J. M., *et al.*, *Proc. Amer. Soc. Hort. Sci.*, **42**, 123 (1943).
[6] East Malling Res. Sta., Ann. Rep. (1959).

RADIOBIOLOGY

Reduction of the Radioactive Content of Milk by a Feeding Procedure

A method for reducing the fall-out content of milk has been investigated. This is a preliminary report on the results obtained in a short-term study in this area. There are to-day commercially available 'grass incubators' which hydroponically germinate various grains to be used as a substitute for fresh pasturage. It is claimed that the product of these incubators fed to cattle on dry lot maintains the cows in a high production of superior-quality milk to an extent which makes the purchase of such incubators economically desirable. The increased use of these feeding systems in this area raised the question to us concerning the effect this substituted diet may have on the radioactive content of milk.

In the experimental work a total of twenty dairy cows was used, all of which had been under the same conditions for several weeks prior to the experiment. The cows were divided into two groups of ten animals each; the control group was kept on natural pasture during the experiment while the experimental group was confined in dry lot and had only incubator-germinated oats as their source of green pasturage. These oats were allowed to germinate for 6 days in the incubators under hydroponic culture and then were fed as intact mats of seed and shoot to the cows. Cultivation was according to the manufacturer's directions (Buckeye Incubator Co., Springfield, Ohio). Each cow in the experimental group was fed 20 lb. of incubator feed daily. Both groups were fed their usual hay and grain diet. Equal aliquots of milk were taken from each cow at each milking and pooled into a weekly sample for each group. Thus the analyses for radioactive content were made on weekly aliquots of pooled samples from each of the two groups. The total volume of each sample was measured and a 4-l. aliquot evaporated to 2 l. in a sand-bath. The residual liquid was counted for cæsium-137 and potassium-40 content. The counting was performed with an 8 in. × 4 in. sodium iodide (Tl) crystal feeding into a 256 channel analyser (Vanderbilt University Low Level Whole Body Counting Facility supported by: United States Atomic Energy Commission Grant No. *AT* (40-1)-240 and Contract No. *DA*-49-007-*MD*-995 with the Army Medical Research and Development Command). The samples were counted for 1 hr., which made possible net counts for cæsium-137 and potassium-40 in the range 500–3,000 counts. The results are given in Table 1 as net counts which are the gross counts less background. Background was run on a solution of 12·5 gm. potassium chloride in 2,200 ml. distilled water, to approximate the potassium content of milk.

The cæsium-137 counts in all of the seven weekly groups were significantly lower (more than twice the standard error of the difference) than the controls, with an average reduction of 38 per cent. The potassium-40 counts were significantly different only in the first and third weekly samples and fail to show a consistent trend.

The differences in the cæsium-137 counts between the control and the experimental groups becomes more significant when we consider that the substituted incubator feed comprises only a part of the total diet. A more detailed study is proposed.

We are indebted to Mr. George Ivey, of Little River Farms, Alpharetta, Georgia, for supplying the cattle and dairy facilities for this pilot study. Also, we wish to express our appreciation to Dr. George R. Menelly and Dr. Robert T. Lagermann for use of the 'Low Level Whole Body Counting Facility' at Vanderbilt University and to Mr. William Van Willis for his technical assistance.

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Radiosensitivity of the Pre-irradiated Yoshida Sarcoma

Acquired radioresistance of cancer has been discussed clinically and experimentally by several authors[1–5]. The various opinions concerning its mechanism may be summarized as follows: (1) selection of cancer cells; (2) induction of mutation and its adaptation; (3) connective tissue response or tumour bed response. Experimental confirmations are less pronounced than clinical observations. One[1,2] of the recent experiments showed development of radioresistance using repeated frequent X-ray irradiations of intramuscular implantation of Ehrlich carcinoma, whereas others[3] noticed no change of radiosensitivity following frequent whole-body γ-ray irradiations of mice bearing Ehrlich ascites carcinoma. The results of the latter may be due to insufficient dose to develop a radioresistance.

It seemed, therefore, of interest to conduct an experimental study of radiosensitivity on the hypothesis that the most suitable way to induce radioresistance is to expose tumour cells to such a dose of irradiation that only a few cells could survive. By repeating this procedure to successive generations radioresistance might be obtained.

Table 1

Sample (weeks)	Cæsium-137 counts				Potassium-40 counts			
	Experimental	Control	Difference	Change (per cent)	Experimental	Control	Difference	Change (per cent)
0*	721 ± 99	732 ± 99	11 ± 140	1·5 ± 19	1,881 ± 84	1,868 ± 84	−13 ± 117	−0·7 ± 6
1	375 ± 97	930 ± 100	555 ± 139	60 ± 15	1,719 ± 83	2,175 ± 85	456 ± 119	21 ± 6
2	377 ± 97	713 ± 99	336 ± 139	47 ± 20	1,640 ± 82	1,664 ± 82	24 ± 116	1 ± 7
3	735 ± 99	1,060 ± 101	325 ± 141	31 ± 13	2,365 ± 86	2,059 ± 84	−306 ± 121	−13 ± 5
4	778 ± 99	1,158 ± 101	380 ± 141	33 ± 12	1,579 ± 80	1,569 ± 80	−10 ± 112	−0·8 ± 7
5	1,069 ± 101	1,667 ± 108	598 ± 147	36 ± 9	2,155 ± 86	1,841 ± 84	−314 ± 119	−15 ± 6
6	1,052 ± 100	1,499 ± 105	447 ± 145	30 ± 10	1,770 ± 83	1,995 ± 85	225 ± 118	11 ± 6
7	818 ± 99	1,117 ± 103	299 ± 143	27 ± 13	1,951 ± 85	1,811 ± 89	−140 ± 118	−7 ± 6
Average 1–7	743 ± 105	1,163 ± 111	420 ± 154	38 ± 14	1,883 ± 89	1,873 ± 89	−10 ± 126	−0·4 ± 7

* This sample was taken before the experimental animals were removed from pasture grass. These values were not included in the calculation of averages.

The experiment was initiated in 1955 using the Yoshida sarcoma. Mixed-bred rats weighing about 100 gm. were used. For the first transfer generation, 5×10^6 tumour cells were transplanted into the peritoneal cavity, and 4 days later this tumour-bearing rat received a whole-body X-irradiation of 1,000 r. Physical factors were 185 kVp., 6 m.amp., half-value layer 0·76 mm. of copper, focus-peritoneal cavity distance 23 cm. and dose-rate 74·1 r./min. in air. 1 hr. later, the same number of tumour cells was transplanted from this rat into the peritoneal cavity of the other rats to make the 2nd generation. After the proliferation of tumour cells at the 2nd generation had occurred in the peritoneal cavity, whole-body irradiation was repeated. 1 hr. later the same number of tumour cells from the 2nd generation rat was transplanted intraperitoneally, making the 3rd generation. Thus, repeated irradiations were carried out up to the 94th transfer generation. The dose was 1,000 r. each for the tumour-bearing rats of the 1st to 31st generation, and 2,000 r. each for those of the 32nd to 94th generation. The tumour cells of the pre-irradiated line were given an integral dose of 87,480 r.

X-ray irradiation of 2,000 r. appeared to be an almost lethal dose for the tumour cells of this type. Five to fifteen rats were used for each transplantation following irradiation. Those transplantations frequently failed. When successful, 7–18 days were required for tumour cell proliferation in the peritoneal cavity after transplantation. In the non-treated control line, on the other hand, 3–4 days were sufficient for the proliferation. Judging from the results[6] of a single cell inoculation of the Yoshida sarcoma into the peritoneal cavity, it is assumed that a few tumour cells remain to proliferate after X-irradiation of 2,000 r.

In order to compare the radiosensitivity of the tumour cells of the pre-irradiated line with that of the non-irradiated line, the cells of both sublines were transplanted subcutaneously in the right shoulder of rats. The tumour regression of both lines following local irradiation with 1,000 r. was studied. The factors of the irradiation were 185 kVp., 6 m.amp., half-value layer 0·76 mm. of copper, focus-tumour distance 13 cm., radiation field 4 cm. in diameter and dose rate 232 r./min. in air. The growth-rate of the tumours was studied by measuring size of tumours, regarding them as ellipsoids in shape. No difference was observed between two sublines. The grade of tumour-regression was higher for the pre-irradiated line than for the non-irradiated line following local irradiation with 1,000 r. This characteristic remained unaltered for 4 months without being exposed to X-rays. The mean values of the tumour size of both lines are shown in Fig. 1. Regression curves were statistically determined and significant difference was found between both sublines. The rate of cure of the subcutaneously transplanted tumours of the pre-irradiated line following local irradiation was significantly higher than that of the non-irradiated line.

From these results, it might be concluded that the pre-irradiated line is not more radioresistant, but more radiosensitive, than the control. It is quite conceivable that irradiation itself induces a mutation which increases the radiosensitivity of tumour cells. From the results of this experiment, it may be inferred that some of the homogeneous population of tumour cells may probably have undergone some mutational changes during the repeated heavy irradiations with X-rays and that these mutant cells proliferate as altered stem-cells. One of the biological characteristics of the pre-irradiated line is that tumour cells involved are more radiosensitive than those in the original line. The fact that selection of tumour cells was not observed may indicate that the individual tumour cell is almost equal in radiosensitivity in the Yoshida sarcoma.

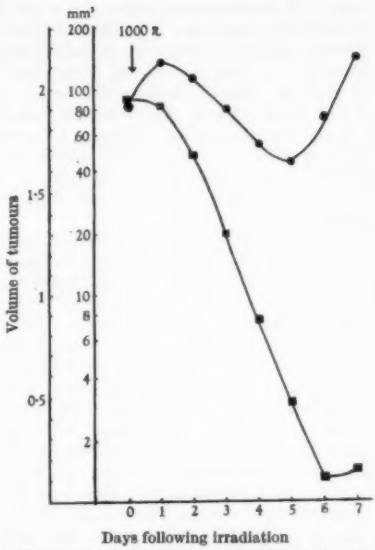

Fig. 1. Changes in volume of subcutaneously implanted tumours following local irradiation with 1,000 r. ●—●, Non-irradiated control line ($y = 1{\cdot}73514 - 0{\cdot}200679\ (x - \bar{x}) + 0{\cdot}00752355\ (x - \bar{x})^2 + 0{\cdot}0150303\ (x - \bar{x})^3$). ■—■, Pre-irradiated line ($y = 0{\cdot}860190 - 0{\cdot}459041\ (x - \bar{x}) + 0{\cdot}0155764\ (x - \bar{x})^2 + 0{\cdot}0178113\ (x - \bar{x})^3$)

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[1] Schubert, G., *Z. Krebsforschung*, **60**, 216 (1954).
[2] Dittrich, W., Höhne, G., and Schubert, G., in "Progress in Radiobiology", ed. by Mitchell, Holmes and Smith, 381 (Oliver and Boyd, 1955).
[3] Conger, A. D., and Luippold, H. J., *Cancer Res.*, **17**, 897 (1957).
[4] Brues, A. M., "Adv. Canc. Res.", **2**, 190 (1954).
[5] Windholz, F., *Radiol.*, **48**, 398 (1947).
[6] Sato, H., *Gann*, **43**, 1 (1952).

BIOLOGY

Transfer of Germ-Cells in *Xenopus laevis*

THE work of Bounoure[1] and Blackler[2] on three anuran species has shown that, in fixed and sectioned material, and after the application of suitable stains, the embryonic sites that contain primordial germ-cells may be recognized at all developmental stages from the fertilized egg to the tadpole. A technique for the transfer of the gonocytes between one embryo and another has been devised as a first step in a genetical analysis of abnormal embryos produced in this department by the transplantation of somatic nuclei into unfertilized eggs of *Xenopus*.

Three stages of development offer the most likely chances of successful transfer. The first of these is at the late blastula stage, in which the primordial germ-cells are localized in the superficial blastocœl floor. Second, at neurula stages, where the cells can be found in the ventral part of the posterior endoderm, and finally, at the stage 40 tadpole (Nieuwkoop and Faber's table[3]) in which the gonocytes are situated at the dorsal crest of the endoderm. Most successful transfers have so far been achieved at neurula stages.

The technique involves the attempted sterilization of the host embryo (though complete sterilization is not absolutely necessary for our purposes) by removal of its germ-cell site, and the replacement of this by the identical site from a donor embryo. Of course it is necessary to ensure that there is no possibility of confusing graft cells with any host germ-cells remaining after an unsuccessful or only partially successful sterilization. In this respect the use of the Oxford nuclear marker, discovered by Elsdale, Fischberg and Smith[4], has proved invaluable. In normal *Xenopus* embryos the nuclei of both somatic and germ-line cells possess potentially two nucleoli, though in some of the cells these nucleoli may be fused. Embryos bearing the mutation affecting nucleolar number possess but a single nucleolus in all nuclei. Thus when normal germ-cells are grafted into 1-nucleolate embryos and the host embryonic gonads later examined in microscopical sections, any cell present the nucleus of which bears two nucleoli must necessarily be of graft origin.

The method of transfer consists in decapsulating host and donor neurulæ in Niu and Twitty solution, excising the posterior endoderm of the host ventral to the archenteric cavity (together with its overlying ventral mesoderm and ectoderm) and replacing it by the same region taken from the donor embryo. The complete obliteration of the wound cuts by ectodermal overgrowth takes a variable time but operated embryos may eventually be transferred to 0·1 Niu and Twitty, in which solution they can be left for a couple of days. About 40 per cent of the operations are successful in that the graft heals in and the host embryo does not develop abnormally.

Results achieved so far have demonstrated clearly the presence in host gonads of grafted germ-cells, though the marker does not allow any conclusion to be reached in terms of 1-nucleolate germ-cells, which may be of host or graft origin. The latest stages to which these results apply at present are metamorphosed frogs in which, in females, the process of oocyte growth has begun.

Thus primordial germ-cells can be successfully grafted from one individual to another. However, it is not sufficient to prove only this point in establishing the technique, for the method is only of practical use if the grafted germ-cells are capable of giving rise to definitive and functional sex-cells. But if they do, then a central question inherent in the germ-cell problem in vertebrates will be settled, namely, as to whether or not primordial germ-cells give rise to definitive gametes. Such a result would be even more unassailable than the striking indications of germ-cell continuity arising from Mintz and Russell's[5] work on mouse embryos. Host animals are at present being reared to sexual maturity with the view of analysing the transfers by breeding, and it is hoped to publish a full account of the technique at a later date.

I wish to thank Dr. M. Fischberg for determining the nucleolarity of the embryos used. The work is being supported by a grant from the British Empire Cancer Campaign.

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[1] Bounoure, L., *Ann. Sci. Nat.*, 10e Ser., **17**, 67 (1934).
[2] Blackler, A. W., *J. Embryol. Exp. Morph.*, **6**, 491 (1958).
[3] Nieuwkoop, P. D., and Faber, J., "Normal Table of *Xenopus laevis*" (North-Holland Pub. Co., 1956).
[4] Elsdale, T. R., Fischberg, M., and Smith, S., *Exp. Cell. Res.*, **14**, 642 (1958).
[5] Mintz, B., and Russell, E. S., *J. Exp. Zool.*, **134**, 207 (1957).

Dermal Surface of Sheep-skin

In the dermal surface of sheep-skin a natural feature has been noticed, which is the origin of a defect in the appearance of some gloving leathers. During manufacture of leather the epidermis is removed, the exposed surface becoming the 'grain' of the leather, and small dark spots, scattered several mm. apart, occur on the grain surface of some leathers. A spot is illustrated in Fig. 1 (top), which shows the surface of a leather made from an Abyssinian sheep-skin. The rows of dark regions represent

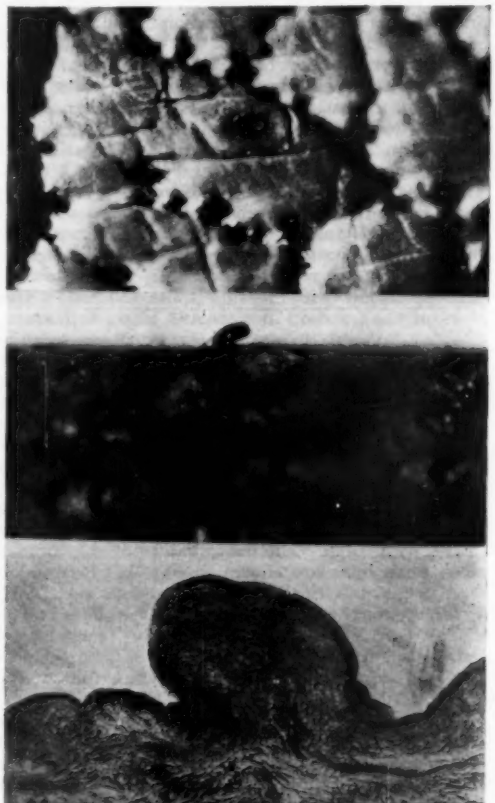

Fig. 1. Top, grain surface of gloving leather showing defective spot between two rows of hair follicle mouths (× *c.* 30); middle, 'tongue' rising from the grain surface of gloving leather (× *c.* 27); bottom, section of the base of a 'tongue' showing it to be a fibrous continuation of the dermis (× *c.* 188)

the mouths of hair follicles and an offending spot lies between two such rows. Microscopical examination of cross-sections showed that the spot was not due to diseased or scar tissue, but that the surface connective tissue fibres had been damaged and were, in the leather, stuck together to give a glassy appearance to the spot. The examination of some other leathers of similar type, the origin of which was, however, not precisely known, gave a lead as to the cause of the spots. Fig. 1 (middle) shows a small tongue of tissue rising from the grain surface: the camera is looking along the grain surface so that the tongue is seen in profile. Fig. 1 (bottom) shows the base of a tongue in cross-section: it is fibrous and continuous with the general fibrous tissue of the dermis. The distribution of tongues was similar to that of the spots, and it seems reasonable to conclude that they rub off during processing, leaving the unsightly spots.

Similar spots or tongues have been observed, so far, in leathers from Abyssinian, Cape, Welsh Hill and Australian Merino sheep-skins.

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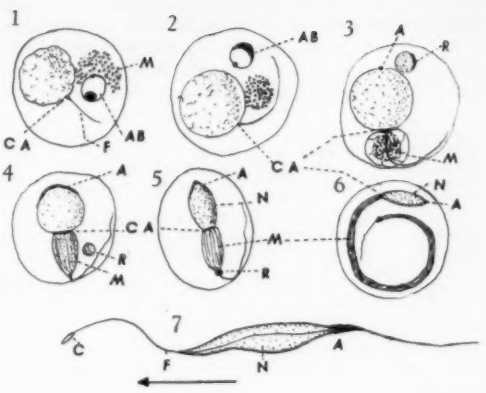
Position of the Proximal Centriole in Flagellate Spermatozoa

What must be one of the most remarkable spermatozoa of Insecta is that of *Lepisma*, as reported by Bowen[1], Nath and Bhatia[2], and Nath[3]. In Fig. 7, this spermatozoon is shown with a centriole (*C*) as its anterior end, connected by a flagellum (*F*), across the nucleus (*N*) to another body (*A*) said to be the acrosome, from which passes back the tail of the spermatozoon. With the acrosome in such a peculiar position, it is difficult to understand how this spermatozoon gets into the egg, always assuming that the acrosome of *Lepisma* takes the usual part in this process. It would seem also that the centriole lies in an unsheltered position, assuming again that this centriole is necessary for initiating the fertilization spindle of *Lepisma*.

We have recently studied the spermiogenesis of another thysanuran, *Petrobius maritimus*, and again are unable to support the interpretation given by Bowen and Nath. Nor do we believe that two insects so closely allied could have spermatozoa basically different in construction. In *Petrobius*, the acroblast (*AB*, Fig. 1) swells up in the early spermatid, producing the acrosome (*A*, Fig. 3), and afterwards flows down as the reject (*R*), which becomes attached for a time to the base of the mitochondrial nebenkern (*M*, Fig. 5). In Fig. 1 the flagellum (*F*) arises from a centriole-like body (*CA*) and curls around inside the cell; the body (*CA*) later forms a flat stainable plate (*CA*) between the nucleus and the mitochondrial middle piece (Fig. 4). This we believe to consist of an anterior centriole, and the centriole adjunct or neck body packing. The acrosome (*A*) lies in its normal position at the head end of the ripening spermatozoon (Fig. 6 *A*). No movement of the centriole forwards has been seen to occur in *Petrobius*. The question of the nature of the plate (*CA*) cannot be solved in such small cells by optical microscopy,



but the formation of the acrosome shown in Figs. 1–3 is quite normal, is within the powers of optical microscopy, and conforms to what has previously been seen in insect spermiogenesis. In some of our preparations, the nucleus between stage Figs. 4 and 5 appeared to have a lateral fold which we were unable to interpret, but which may be Bowen's supposed forward continuation of the flagellum (*F*, Fig. 7). Thus we consider that in Fig. 7, after Bowen and Nath, the anterior bead (*C*) is not the centriole, but is the acrosome (whole or part), whereas the body (*A*) is the adjunct plus the centriole, as has recently been shown to be the case in Orthoptera and Coleoptera by Gatenby and Tahmisian[4], who used the higher powers of the electron microscope.

In stages of spermiogenesis later than Fig. 6, the ripening sperm is seen to have double spiral threads passing down around the flagellum. The sperm is very small; but we could not find any reason to believe, as did Nath, that this spermatozoon is bereft of mitochondrial substance.

In his useful review on the cytology of spermatogenesis, Nath[3] discusses various "atypic flagellate sperms with centriole at the anterior tip". All such cases need re-investigation with the electron microscope, and until this has been carried out, we are inclined to believe that such "atypic flagellate spermatozoa" have no existence in the animal kingdom. Another statement which we find nowadays difficult to accept is that the acroblast directly metamorphoses into the acrosome, that is, "the direct method of acrosome formation" according to Nath and a few previous authors.

So far as we are able to understand, all recent work with the electron microscope supports the view that the acroblast is a pile of lamellæ (or more rarely a series of lamellated vesicles) which in some way concentrates the material included in the acrosome. It does not seem easy to believe that this pile of lamellæ changes into a globular electron translucent acrosome. On the contrary, the lamellæ always seem to be rejected as the Golgi remnant.

References to a centriole at the anterior end of the spermatozoon occur sporadically in the immense literature of the optical microscopy of animal spermiogenesis; but such recent electron microscopists as Burgos and Fawcett[6], Grassé, Carasso and Favard[7], Afzelius[8], Yasuzumi and Tanaka[9], Gatenby and Dalton[10], Gatenby and Tahmisian[4], who worked on mammals, molluscs, insects, oligochætes or sea-

urchins, all found the proximal centriole in its normal post-nuclear position in the neck of the spermatozoon.

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[1] Bowen, R. H., *J. Morph. Physiol.*, **39**, 351 (1924).
[2] Nath, V., and Bhatia, C. L., *Res. Bull. East Panjab Univ.*, **27**, 33 (1953).
[3] Nath, V., *Internat. Rev. Cytol.*, **5**, 395 (1956).
[4] Gatenby, J. Brontë, and Tahmisian, T. N., *La Cellule*, **59**, lx (1959).
[5] Gatenby, J. Brontë, and Mukerji, R. N., *Quart. J. Micro. Sci.*, **73** (1929).
[6] Burgos, M. H., and Fawcett, D. W., *J. Biophys. and Biochem. Cytol.*, **287** (1955).
[7] Grassé, P.-P., Carasso, N., and Favard, P., *Ann. Sci. Nat. Zool.*, **339** (1956).
[8] Afzelius, B., in Almquist and Wiksell, "Boktryck" (Uppsala, 1957).
[9] Yasuzumi and Tanaka, *J. Biophys. and Biochem. Cytol.*, **4**, 621 (1958).
[10] Gatenby, J. Brontë, and Dalton, A. J., *J. Biophys. and Biochem. Cytol.*, **6**, 45 (1959).

Mitochondria of Goldfish, *Carassius auratus*

The metabolism of mitochondria of poikilotherms acclimatized at different temperatures has not been studied, though such studies have been made on rats[1,2]. The mechanisms of adaptation to cold and warm temperatures in a poikilotherm may be different from those of a homoitherm due to the presence of a temperature-regulating centre in the latter. Hence, mitochondria from livers of goldfish, *Carassius auratus*, acclimatized at 10° and 30° C. were isolated to study their oxidative phosphorylation. The following account gives the method of isolation and the structure of mitochondria of the liver of unacclimatized goldfish.

The liver was placed in ice-cold 0·25 *M* sucrose solution immediately after its removal from the goldfish. All the following steps for the isolation of mitochondria were conducted at 0°–2° C. 2 gm. of the liver were homogenized in 10 c.c. of 0·25 *M* sucrose (buffered at *p*H 7·4 with *tris* buffer and containing 0·001 *M* versene) by fifteen up-and-down strokes of a Potter–Elvehjem homogenizer driven at 300 r.p.m. The homogenate was centrifuged at 700*g* for 10 min. in a refrigerated centrifuge to remove the cellular debris and nuclei. Then the supernatant was pipetted out and centrifuged at 14,000*g* for 10 min. to sediment the mitochondria.

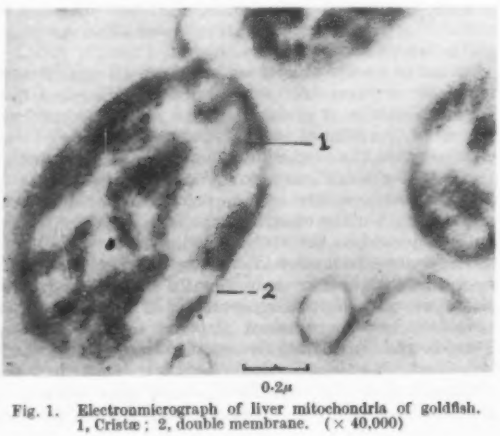

Fig. 1. Electronmicrograph of liver mitochondria of goldfish. 1, Cristæ; 2, double membrane. (× 40,000)

Electronmicrographs of the mitochondrial pellets showed that the mitochondria of the liver of goldfish obtained by the above method were intact (Fig. 1). They were oval in shape, 0·8–1·0μ in length and 0·5–0·6μ in breadth. They had a double membrane and cristæ as in rat liver mitochondria. These mitochondria stained well with janus green.

Oxidative phosphorylation of the liver mitochondria of goldfish acclimatized at 10° and 30° C. will be discussed elsewhere. I am grateful to Prof. C. L. Prosser, Department of Physiology, University of Illinois, for his guidance.

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[1] Panagos, S., Beyer, R. E., and Masoro, E. J., *Biochim. Biophys. Acta*, **29**, 204 (1958).
[2] Smith, R. E., and Fairhurst, A. S., *Proc. U.S. Nat. Acad. Sci.*, **44**, 705 (1958).

Spread of the Ascidian, *Styela mammiculata* Carlisle

Carlisle[1] described a new species of ascidian, *Styela mammiculata*, which was first taken in 1953, near Plymouth. If, as Carlisle suggested, this is an introduced species, it may be expected to spread. The discovery of numerous specimens on various submerged surfaces and in dredged material in Langston Harbour, Hampshire, during the summer of 1959, is therefore of interest. The species appears to be extending its range rapidly along the south coast of England, and increasing in numbers where already established. We expect it to spread to other sheltered and warm areas of the British and continental coast, either by natural means or by transport on the hulls of ships.

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[1] Carlisle, D. B., *J. Mar. Biol. Assoc. U.K.*, **33** (2), 329 (1954).

FOREST PRODUCTS

Effect of Moisture Changes on Creep in Wood

During studies on creep in wood under various types of loading over periods of up to several years, it became apparent that changes in the moisture content of the wood while under load markedly influenced both creep-rate and total creep.

To explore this further, small beams of ¾-in. square cross-section were cut from billets of three species, namely, *Eucalyptus regnans* (a hardwood prone to collapse of the cell wall during seasoning), *Eucalyptus pilularis* (a hardwood with very little tendency to collapse), and *Pinus radiata* (a softwood with negligible tendency to collapse). Three moisture conditions were studied, namely, green material maintained in the green state during test, initially green material allowed to dry while under load, and material air-dried to 12 per cent moisture content before loading and maintained at approximately that value during test.

The tests were conducted in a temperature-controlled atmosphere at 25° C. The beams were simply

supported and subjected to four-point loading for a period of approximately two weeks. During this time the moisture content of the wood that was allowed to dry had reached a reasonably constant value. Unloaded beams of matched material were used as controls at each moisture condition. The maximum deflexions of the beams relative to the points of support, and the strains in the extreme fibres on the compression and tension faces near the centre of the span, were measured at intervals during test.

The results showed that the relative creep in wood allowed to dry while under load was at least twice that occurring in wood maintained at constant moisture content. Relative creep is defined as the ratio of creep deformation to initial deformation. Further, the relative creep was approximately the same in wood maintained at constant moisture content irrespective of whether it was green or dry. The strains in the extreme fibres were, for all three moisture conditions, much greater on the compression faces of the beams than on the tension faces, the maximum values of extreme fibre strains in both compression and tension being obtained for material drying under load. Similar results were obtained for each of the species tested.

Exploratory experiments have indicated that creep also increases markedly in wood subject to increasing moisture content during test, and that the effect of moisture changes on stress relaxation in beams held at constant deflexion show similar trends (P. U. A. Grossman, personal communication).

In the case of green wood drying while under load a marked difference in total creep and creep-rate has been observed in geometrically similar beams of different sizes. The average drying-rates for the sizes tested differed considerably and it would be expected that the restraints due to moisture gradients in the beams would have been different. Experiments are in progress to determine to what extent the rate of loss or gain of moisture affects the creep-rate and total creep.

In addition, tests have been commenced to determine the effect on creep of small changes in the moisture content of dry wood.

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PATHOLOGY

Urinary Selenium and Dental Caries

The suggested direct relationship between urinary selenium concentrations and the prevalence of dental caries in American children[1,2] is not substantiated by an investigation in New Zealand. Until recently, studies on the physiological effects of selenium have been concerned with its toxicity. Later evidence suggests that for some animals, and at certain levels, the element may be classified as essential[3]. Although selenium has not been demonstrated to be essential in man, its suspected role in electron transport may suggest a possible modification of the calcified structures. In New Zealand, the marked improvement in health, weight gain, and reduction of muscular dystrophy in sheep ('white muscle disease') by dosing with selenium[4], suggests there is a minimum level of available selenium in many areas of New Zealand. This evidence warranted an investigation of the supposed selenium–caries relationship.

Analytical data obtained in this investigation, and other work to be published elsewhere, indicate the low levels of available selenium.

The investigation was carried out in two rural areas of the South Island. Both areas are of different soil types, one being of yellow-grey and the other of yellow-brown earths. In neither district has selenium deficiency been reported in farm animals.

In South Otago, 39 school boys 5–14 years old were examined for dental conditions, and 31 of similar ages were examined in North Canterbury. The children were life-time residents of their districts.

All dental examinations were carried out by the one examiner (P. B. C.), and were supplemented by bitewing radiographs. 24-hr. samples of urine were collected under parental supervision, from the children concerned. Samples were collected in polythene bottles containing toluene as a preservative. Every practicable care was taken to ensure the reliability of the samples.

Selenium analysis was carried out by a modification of the colorimetric diamino-benzedine method of Luke[5]. Recoveries were of the order of 94 per cent. Readings were made on Beckman model *DU* spectrophotometer.

Selenium was present in all urine samples. In Table 1 the mean values of urinary selenium-levels for both groups are lower than those in the four areas investigated by Hadjimarkos[1,2], and are believed to be indicative of low selenium availability. The difference of the means for the districts is significant at the 99 per cent level of confidence.

Table 1. Distribution of Urinary Selenium-Levels

Urinary selenium (p.p.m.)	Number of male subjects 5–14 years old in : South Otago	North Canterbury
0·00–0·09	2	0
0·10–0·19	18	3
0·20–0·29	13	11
0·30–0·39	5	15
0·40–0·49	1	2
Totals :	39	31
Means :	0·021 p.p.m.	0·030 p.p.m.
Standard deviation :	± 0·009	± 0·008

It has been shown that the rate of urinary excretion is relatively constant, and is quite independent of urinary volume[6]. As the concentration of selenium is a function of the volume excreted, the volume being subject to climate, fluid intake, and other factors, it is felt that a more reliable basis of comparison may be given by the total urinary selenium excreted in 24 hr.

Table 2 gives the amount of selenium excreted by subjects in the two districts in terms of μgm./24 hr. The mean excretions in each district are significantly different at the 98–99 per cent level of confidence.

The percentage of available tooth surfaces decayed and filled was 22·6 at South Otago and 18·8 at North Canterbury. The difference of 3·8 has a standard error of 9·8. Therefore, the difference is not significant.

There was no correlation between the proportion of available tooth surfaces decayed and filled and the total urinary selenium excreted in 24 hr. (Correlation coefficients were plus 0·019 for South Otago, and plus 0·06 for North Canterbury. Standard errors of the correlation coefficients were 0·16 and 0·16, respectively).

Table 2. Selenium Excreted in μgm./24 hr.

Selenium (μgm./24 hr.)	Number of male subjects 5–14 years old in: South Otago	North Canterbury
0–9	5	1
9·1–18	21	10
18·1–27	9	11
27·1–36	3	6
36·1–45	0	1
45·1–54	0	0
54·1–63	1	2
Totals	39	31
Means	17·0 μgm.	23·7 μgm.
Standard deviation	± 9·8	± 12·2

In this investigation the significant difference in selenium excretion between groups was not associated with any significant difference in caries prevalence.

This investigation was carried out as a project of the dental research unit of the Medical Research Council of New Zealand. The assistance and advice of Captain F. L. Losee (D.C.), U.S.N., is gratefully acknowledged.

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[1] Hadjimarkos, D. M., and others, *J. Pediatrics*, **40**, 451 (1952).
[2] Hadjimarkos, D. M., and Bornhorst, C. W., *J. Pediatrics*, **52**, 274 (1958).
[3] Schwarz, K., and Doltz, C. M., *J. Amer. Chem. Soc.*, **79**, 3292 (1957).
[4] Hartley, W. J., *N.Z. J. Agric.*, **99**, 259 (1959).
[5] Luke, C. L., *Anal. Chem.*, **31**, 572 (1959).
[6] Sterner, J. H., and Lidfeldt, V., *J. Pharm. Therap.*, **73**, 205 (1941).

Cementing Substances in Metastasizing and Non-Metastasizing Transplantable Tumours in Mice

Very little is known about whether, or how far, the condition of intercellular cementing substances as present in the tissue of a growth may be responsible for local invasion and for metastasis[1,2]. The situation is all the more complex as neoplastic cells may even themselves produce polysaccharides[3], which are considered to be the fundamental chemical constituents of the intercellular cement. The condition of the cementing substances in 13 transplantable tumours in mice have been studied in this laboratory. Results are summarized in Table 1.

Primary tumours were fixed in fluids containing alcohol, formalin and picric and acetic acids, preferably at low temperature (Duboscq–Brasil and Gendre). Particular emphasis was placed on selecting non-necrotic portions for microscopical study. Several staining and histochemical procedures for identifying mucopolysaccharides were used, the most useful being the Hale[4,5] and the periodic acid–Schiff reactions. Phloxine and hæmalum were also occasionally used for cytoplasmic and nuclear counterstaining. The latter removes, however, the Hale-positive cement of epitheliomas and should not be employed in neoplasms of this type.

(1) *The ground substance in eight transplantable sarcomas.* The greatest quantity of the Hale-positive material was present in the ground substance of non-metastasizing tumours (see Table 1, 1–4). There was in these tumours also a great amount of alcian-blue-positive material (not given in Table 1). On the other hand, the Hale-positive material was absent in highly and moderately metastasizing tumours (Table 1, 7 and 8); the quantity was intermediate in slightly metastasizing tumours (Table 1, 5 and 6). In other words, metastatic capacity increases when Hale-positive material diminishes. By contrast, the periodic acid–Schiff-positive material was absent in most of non-metastasizing tumours (Table 1, 1, 2 and 3) and present in most of metastasizing tumours (Table 1, 5, 6 and 8). But there were definite exceptions from this rule (Table 1, 4 and 7). These exceptions are most probably due to the technique, which does not prevent a certain degree of extraction of the more soluble components of periodic acid–Schiff.

Digestion by hyaluronidase and metachromatic staining tend decidedly to favour the assumption that Hale-positive material is chemically related to mucopolysaccharides, at least in certain non-metastasizing sarcomata.

(2) *The intercellular cementing substances in five epitheliomas.* The antimetastatic influence of Hale-positive material was again fully evident in epitheliomas (Table 1, 9–13). On the other hand, metastatic capacity was the more pronounced the greater the quantity of periodic acid–Schiff (compare Table 1, 9 with 10–13). A moderate amount of a similar

Table 1

No.	Tumour*	Strain of mouse	Metastatic capacity	Amount of Hale-positive material in the: Ground substance‡	Intercellular cement‡	Cytoplasm	Amount of periodic acid – Schiff in the: Ground substance	Intercellular cement	Cytoplasm
	Sarcomas								
1	*Sa*†	*DBA*/1	none	++++		+	–		trace
2	*Sb*†	*DBA*/1	none	++++		+	–		—
3	*GL*46 (early transfer)	*DBA*/1	none	+++		trace	–		trace
4	*D*3†	*C*3*H*	none	+++		trace	++		trace
5	*GL*46 (later transfer)	*DBA*/1	slight	++		trace	+		trace
6	*S*180	*C*57*BL*/10*d*	slight	+		—	trace		trace
7	*D*1†	*C*3*H*	moderate	–		trace	–		trace
8	*T*241	*C*57*BL*	high	–		trace	+		trace
	Epitheliomas								
9	*Pch* (solid)†	*CA*58	slight		+	++		–	–
10	*K*7†	*C*3*H*	moderate		+	+		+	–
11	*K*9†	*C*3*H*	moderate		+	+		+	–
12	*Pch* (ascitic)†	*CA*58	moderate		trace	trace		+	++
13	*T*150	*DBA*/1 or *Lp*	high		–	–		+	trace
12′	*Pch* (ascitic) (*ip*)†	*CA*58	slight		+	++		trace	+

All epitheliomas, excepting the bladder carcinoma 13, are mammary adenocarcinomas.
* All were grafted subcutaneously. In addition, *Pch* (ascitic) was inoculated intraperitoneally (see 12′).
† Isografts; 5, 6 and 13 are homografts which took in 100 per cent of cases.
‡ Quantities of ground substance in sarcomas and of intercellular cement in epitheliomas are not comparable; comparison is admissible only within the same type of tumours, that is, within the group of sarcomas, or within the group of epitheliomas.

substance could also be seen in the stroma of the more invasive epitheliomas (not mentioned in Table 1), a phenomenon already described by Gersh and Catchpole[2].

(3) *Intracytoplasmic Hale- and periodic acid–Schiff-positive material.* Hale-positive material was in general present in the cells of slightly and non-metastasizing tumours (Table 1, 1, 2, 9 and 12′); it was absent or less abundant in the cells of other metastatic tumours (exception Table 1, 10 and 11). The intracytoplasmic periodic acid–Schiff was observed in diffuse form in all cells, and as granules in some. The granules were apparently absent in tumours 2, 9, 10 and 11.

Cytoplasmic Hale-positive material frequently occupied a concavity of the nucleus (Golgi zone). This is probably the site of synthesis of Hale-positive material, especially in the ascitic form of *Pch* (Table 1, 12′). The concentration of mitochondria around this region is in favour of this assumption, as likewise various morphological signs of metabolic activity of the nucleus: digitations of its membrane and a greater concentration of the chromatin adjacent to the Golgi zone.

In several tumours (Table 1, 1, 2, 9, 10, 11 and 12′) cytoplasmic Hale-positive material seemed to be continuous with that present in the intercellular spaces. A similar continuity of periodic acid–Schiff material was observed only occasionally and irregularly.

Certain observations suggest that the cementing substances are synthesized and secreted rather than absorbed by the tumour cells. This assumption is supported by the extension of the cytoplasmic Hale-positive material from the nuclear indentation to the cell wall, and finally to intercellular spaces, as observed in our tumour 12′ growing intraperitoneally. This may possibly explain why tumours differ as to local invasiveness and metastatic behaviour, both depending seemingly on the quantity and nature of the cementing substances originating in the tumour cells themselves.

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[1] Ambrose, E. J., *The Advancement of Science*, No. 57, 473 (June 1958).
[2] Gersh, I., and Catchpole, H. R., *Amer. J. Anat.*, **85**, 457 (1949).
[3] Meyer, K., "Harvey Lectures", **61**, 88 (1957).
[4] Müller, G., *Acta Histochem.*, **2**, 68 (1955).
[5] Graumann, W., and Clauss, W., *Acta Histochem.*, **6**, 1 (1958).

Relation of Leydig Cells in the Human Testicle to the Tubules and Testicular Function

Groups of Leydig cells have been found in the spermatic cords in 23 of 30 consecutive necropsies. The groups of Leydig cells most commonly occur in the 3 cm. of cord immediately above the upper pole of the testes, but they may be found up to 10 cm. above the globus major, with considerable variation in distribution, both in numbers present and numbers of levels. The cells are constantly distributed in the loose areolar tissue around nerve sheaths and usually infiltrate the nerve bundle, disorganizing the characteristic wavy pattern of nerve fibres. Such cells show all the morphological features characteristic of Leydig cells, crystalloids of Reinke and hyaline bodies occasionally being evident. Infiltration of the nerves by Leydig cells has been described, but not explained, by various authors[1–4]. Some thirty years ago this peculiar association occasioned discussion as to the nature of the cells[5,6]. The relation to nerves can be accounted for either (*a*) by migration along nerve fibres at the embryonic period when nerve fibres grow into testicular parenchyma, or (*b*) by traction of cells from the formed parenchyma during descent of the testicle. The first explanation can be discounted by the similarity of the trabecular pattern of capillaries in normal nerves and the trabecular pattern shown by the infiltrative cells before their continued proliferation disrupts the organized appearance of the nerve.

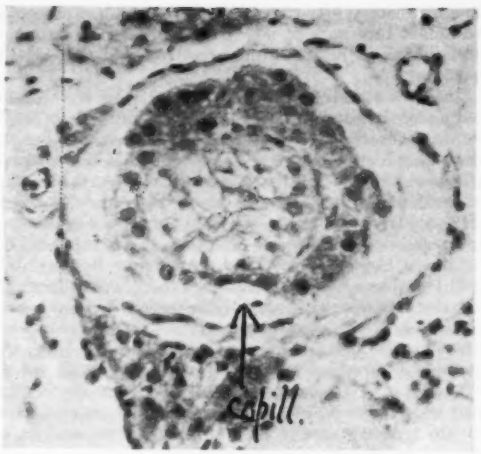

Fig. 1. Hyaline thickening of tubule with residual germinal epithelium and intact inner lamella surrounded by Leydig cells, and with empty capillary spaces at 6 o'clock. (Hæmatoxylin and eosin. × 250)

A similar infiltrative proliferation is seen in hyaline change of the tubular substantia propria where histological detail is clearer, and where active changes take place at the inner lamella, to which are applied thin-walled capillaries. In ordinary paraffin preparations such capillaries are one cell thick and similar to those inside nerve bundles. From the earliest phases of hyaline thickening of the tubule wall, varying numbers of Leydig cells have been seen within the substantia often continuous with groups outside the tubule. In advanced thickening, Leydig cells are typically applied to the inner lamella (Fig. 1). Leydig cells are generally regarded as being an endocrine epithelium and intimate application to thin-walled capillaries is to be anticipated. This association in sites that are abnormal by conventional anatomical standards may be of general biological significance.

In the testes, the intertubular tissue is of scant cellularity, with most of the capillaries present, either in Leydig cell groups or closely applied to the tubule wall. Thus, there are few available capillaries of optimum size along which Leydig cells may proliferate, and the immediate explanation of this infiltrative activity probably is that the capillaries of the invaded structures are the only ones available. This raises

the questions (*a*) why, in Leydig cell proliferation, adequate numbers of new capillaries are not formed, and (*b*) what factors limit growth along the existing vascular tree.

In (*a*) a recent preliminary report[7] on the possible reduction in the number of small blood vessels in the seminal vessels of the castrate rat is in part suggestive that the local action of Leydig cell secretion may effect capillary proliferation. However, the process of infiltration of the hyaline substantia is usually diffusely distributed. In (*b*) if it is assumed that endocrine epithelium must have a thin-walled capillary blood supply, it follows that limitation of proliferation along the capillary bed, away from the tubular capillaries, will be effected by increasing thickness of the vessel wall, be it arteriole or venule. The point of importance in these observations is that the same capillary supplies Leydig cells and tubule.

Because of the extreme tortuosity of the tubules and the close application of many of the Leydig cells to the tubular wall, it is difficult to demonstrate clearly on which side of the tubular capillary bed the Leydig cells are situated. In the spermatic cord the Leydig cells are situated close to the arterioles and the same relations probably exist within the testicular parenchyma.

On the basis of these findings, I advance the hypothesis that Leydig cells are situated between the arterioles and the testicular tubules and have the same capillary blood supply as the tubules. This hypothesis may usefully be applied to several aspects of testicular function, notably the gonadotrophic effect of steroid hormones and their varying effect at different dosage-levels, the irregular segmental distribution of atrophic changes in mature testes, pubertal development and abnormal segmental development as in cryptorchidism, as well as possible mechanisms in male infertility and its therapy.

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[1] Nelson, A. A., *Amer. J. Path.*, **14**, 831 (1938).
[2] Sternberg, W. H., *Amer. J. Path.*, **25**, 493 (1949).
[3] Okkels, H., and Sand, K., *J. Endocrinol.*, **2**, 38 (1940).
[4] Warren, S., and Olshausen, K. W., *Amer. J. Path.*, **19**, 307 (1943).
[5] Brannen, D., *Amer. J. Path.*, **3**, 343 (1927).
[6] Berger, L., *C.R. Acad. Sci., Paris*, **175**, 907 (1922).
[7] Berry, R., *et al.*, *Angiology*, **10**, 372 (1959).

HISTOLOGY

Histochemical Demonstration of Succinic Dehydrogenase Activity in Osteoclasts

A review of the literature has revealed but few studies on enzyme characteristics of osteoclasts. None of these, however, related to dehydrogenase activity.

In the present work frozen sections of rat and hamster knee joints and jaws, 8–10μ thick, were prepared according to the Adamstone–Taylor cold knife technique and mounted on glass microscope slides. In addition, pieces of calvarium from newborn hamsters were also employed. Slides and specimens were then incubated for 60–90 min. in the succinic dehydrogenase substrate solution as described by Pearson[1]. This contained nitroneotetrazolium. Representative sections were also incubated in a substrate solution containing nitro blue tetrazolium[2] as

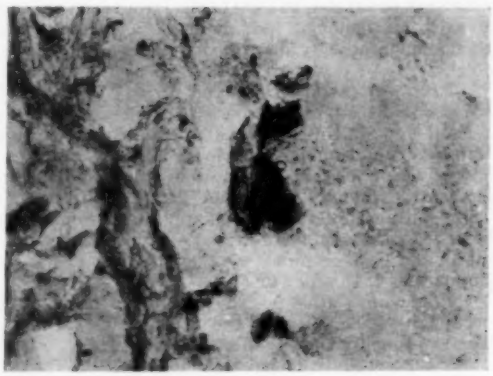

Fig. 1. Succinic dehydrogenase activity of osteoclasts (knee joint from 2-day-old hamster). (× *c.* 214)

described by Nachlas *et al.* With both substrates an intense selective staining reaction was observed in osteoclasts (Fig. 1). Sections incubated in tetrazole without the co-factor (succinate) were unstained, as were heat-treated sections. Sodium malonate was inhibitory. The staining of osteoclasts associated with the remodelling resorption of the jaws was particularly striking. Osteoblasts by contrast showed little if any reaction.

Osteoclasts associated with both intramembranous and intracartilagenous bone resorption showed a similar reaction. The cytoplasm was evenly stained, and it was difficult to assess any mitochondrial reaction.

Osteoclasts have also been observed to exhibit high acid phosphatase[3] and aminopeptidase activity[4]. The present study would further tend to confirm the hypothesis that these cells exhibit high metabolic activity. Localization of succinic dehydrogenase to osteoclasts may be considered as presumptive evidence that the Krebs cycle is involved in the resorptive process.

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[1] Pearson, B., *J. Histochem. and Cytochem.*, **6**, 112 (1958).
[2] Nachlas, M. M., Tsou, K. C., De Souza, E., Cheng, C. S., and Seligman, A. M., *J. Histochem. and Cytochem.*, **5**, 420 (1957).
[3] Burstone, M. S., *J. Histochem. and Cytochem.*, **7**, 39 (1959).
[4] Burstone, M. S., Amer. Assoc. Adv. Sci. Monograph (in the press).

Histochemical Localization of Oxidase Activity in the Mitochondria of the Heart in Some Lower Vertebrates

With the aid of some new methods[1–3] the histochemical localization of oxidase activity was investigated in the mitochondria of the heart in some lower vertebrates, namely, the viviparous cyprinodonts, *Lebistes reticulatus*, *Xiphophorus helleri* and *Xiphophorus maculatus*, the carp, *Cyprinus carpio*, the frog, *Rana temporaria*, the toad, *Bufo calamita* and the lizard, *Lacerta agilis*.

To permit a satisfactory comparison with the results of Burstone[3] obtained with the mitochondria

of the human heart, we applied the corresponding histological technique. Immediately after death by decapitation, 8-μ thick sections of frozen heart muscle were prepared according to the cold-knife method of Adamstone–Taylor, after which they were mounted on chemically clean glass microscope slides. The incubation, which was carried out at 24° C. and lasted ½ hr., was in a substrate solution containing 10 mgm. of variamine blue *B* base or *p*-methoxy-N-phenyl-*p*-phenylenediamine (Carbic Color and Chemical Co., New York) and 10 mgm. of *p*-aminodiphenylamine or N-phenyl-*p*-phenylenediamine (British Drug Houses, Poole, England). At first the substrates were placed in a 50·0-ml. Erlenmeyer flask and dissolved in 0·5 ml. of ethanol reagent. After adding 35·0 ml. of distilled water and 15·0 ml. of 0·2 *M tris* buffer *p*H 7·4 the solution was shaken and filtered through folded filter paper into a Coplin jar. Incubating solutions containing 0·001 *M* sodium sulphide as well as 0·001 *M* potassium cyanide were also used. In addition, the effect of adding per Coplin jar 20·0 mgm. of cytochrome *c* (Sigma Chemical Co., St. Louis, Mo.) to the substrate solution was studied. Some sections were pretreated with physiological saline for half an hour prior to the incubation. Afterwards the sections were incubated in substrate solutions with and without cytochrome *c*. After incubation the slides were stored for 1 hr. in a 10 per cent solution of cobaltous acetate in 10 per cent formalin containing 5·0 ml. 0·2 *M p*H 5·2 acetate buffer. Then the slides were washed for about 5 min. in running water and mounted in polyvinyl pyrrolidone[4] or glycerol-gelatin.

The distribution of the dye with routine incubations appeared to correspond to that found with the classical mitochondrial stains such as the Regaud stain. Just as in the human heart[3] the localization corresponded exactly to those observed for heart sarcomes or mitochondria by Cleland and Slater[5] when using the mitochondrial method of Altmann. Also in our cases the distribution of the staining corresponded very well with the sarcoma pattern as observed under the electron microscope[6]. Some areas showed a perinuclear staining corresponding to the localization of the perinuclear sarcomes.

When the reaction was completely inhibited by addition of sulphide and cyanide, as effected by pre-incubation in physiological saline, it was completely restored in the saline-treated sections by addition of cytochrome *c* to the substrate solution. Moreover, the staining intensity could be augmented to some degree by adding cytochrome *c* to the routine incubation solutions. Consequently, the cytochrome oxidase is believed to be associated with the mitochondria. Just as in the human heart[3], on the strength of the localization of the staining reaction to the mitochondria, the results of the sulphide and cyanide inhibition and the cytochrome *c* tests, one can conclude that cytochrome oxidase occurs in the heart of the lower vertebrates investigated.

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[1] Burstone, M. S., *J. Histochem. and Cytochem.*, **7**, 112 (1959).
[2] Burstone, M. S., *J. Histochem. and Cytochem.* (in the press).
[3] Burstone, M. S., *Nature*, **184**, 476 (1959).
[4] Burstone, M. S., *Amer. J. Clin. Path.*, **28**, 429 (1957).
[5] Cleland, K. W., and Slater, E. C., *Quart. J. Micro. Sci.*, **94**, 329 (1953).
[6] Kisch, B., and Bardet, J. M., "The Electron Microscopic Histology of the Heart" (Brooklyn Medical Press, New York, 1951).

ENTOMOLOGY

Nocturnal Detection of Tsetse Flies in Nigeria with Ultra-Violet Light

Jewell[1,2], in Tanganyika, showed that tsetse flies marked with fluorescent paint can be located at night by means of ultra-violet light resting on leaves. A portable petrol generator was his current supply (personal communication). Because such equipment is cumbersome, other workers[3] have used a hand torch to search for flies painted with reflective glass beads mixed with glue.

The ultra-violet method has been used at the West African Institute for Trypanosomiasis Research to study the nocturnal resting sites of *Glossina palpalis* R.-D. and *G. submorsitans* Newst. in Northern Nigeria. A portable, fairly compact, battery-powered current supply is used. In the case of *G. palpalis*, the information so obtained will form the basis of an experiment on the selective spraying of residual insecticide during the dry season.

The apparatus was supplied by Electric and Musical Industries, Limited, Hayes, Middlesex. A 12-V. car battery feeds d.c. current to a 'Valradio 12/150*B*' convertor which produces a.c. current at 250 volts. After passing through special control gear and the necessary chokes and condensers, this voltage powers two 80-watt ultra-violet bulbs, each in a parabolic reflector on 100 ft. of flex. The entire machine, in a wooden case, fits into a car boot. If no road exists to the study area, the equipment can be broken into two 50-lb. loads, one of which is the battery. These are easily head-carried by labourers. The pigment, a non-radioactive, luminescent powder, was supplied by Barber Medico-electronic Laboratories, Ltd., Weston-super-Mare, Somerset. It adheres well to the thorax of the fly if mixed with thin gum arabic office glue to which has been added a trace of detergent. In the laboratory, painted flies survive as well as unpainted controls. Four colours (blue, yellow, white, orange) are used, permitting sex, time of marking and hunger stage to be identified if desired.

The quantity of light varies with individual bulbs, the best of which concentrate a central beam of ultra-violet sufficient for detecting flies at a distance of 25–30 ft. Sufficient light in the visual range is produced to enable the operator to move about without a torch. The light is attractive to flies disturbed by the operator's movements, and some will fly into the reflector. These can be shaken out and if the lamp is directed away from them, will come to rest nearby. Undisturbed flies, however, do not leave their perches to come to the light, nor does closer inspection with a hand torch disturb them. Some confusion is caused by fireflies (Lampyridae), but these can be identified by their regular flashing. Certain arachnids and fungi fluoresce under ultra-violet, but never as brightly as the painted flies.

Many trials with the riverine species, *G. palpalis*, have revealed that about half the flies rest during the night within 12 in. of the ground, on leaves or small twigs, but never on tree trunks or branches. There appears to be no difference between the sexes in resting height preference at night. Flies released in the morning are rarely found on the night of the same day, having dispersed up- and down-stream in search of food. The best method is to catch the flies in the afternoon, then mark and drop them into a large cage with a removable top. At dusk the cage

top is removed, and those flies that fly out are considered uninjured by handling and marking. 10–35 per cent of marked *G. palpalis* come to rest within the 125-ft. searching radius of the machine, the great majority of them alighting very close to the point of release.

Approximately 50 recoveries of marked specimens of the open woodland species, *G. submorsitans*, in a forest-island breeding site, revealed the flies resting on twigs, leaves and small creepers at heights ranging from 1 to 15 ft., with the majority above 7 ft. It would appear, then, that this species' daytime resting preference for tree trunks, at least when gorged[4], should be considered when planning control by residual insecticides.

Both species' habit of resting on leaves and twigs at night might be a means of avoiding predators such as ants and jumping spiders, the vibration of the predator's approach serving to alert the fly, whereas a branch or tree trunk daytime habitat would permit maximum visibility in the fly's search for a host.

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[1] Jewell, G. R., *Nature*, **178**, 750 (1956).
[2] Jewell, G. R., *Nature*, **181**, 1354 (1958).
[3] Rennison, B. D., *et al.*, *Nature*, **181**, 1354 (1958).
[4] Nash, T. A. M., *Bull. Ent. Res.*, **43**, 33 (1952).

Sex-limited DDT-Resistance in House-flies

A new mechanism of inheritance of DDT-resistance has been investigated in a selected strain of house-flies (*Musca domestica* L.) wherein resistance is manifest only in males. Genetic tests have shown that the resistance is transmitted by males but not by females. It is therefore determined either directly by a *Y*-chromosome factor or, like sex in *Drosophila*, by the balance between the unpaired parts of *X* and *Y* and the rest of the chromosome set.

Evidence for the existence of this patrilineal inheritance dates back to 1954 when an attempt was first made to derive a homogeneous susceptible strain from the Canberra laboratory colony[1] which, although bred since 1939 in isolation from insecticides, had been found to contain a DDT-resistance factor in a small proportion of both males and females. Selection for early maturation (giving strain *E*) eliminated resistant females by the sixth generation, but the strain continued to produce some resistant males which, during a further 68 generations, increased in proportion to about 75 per cent of males produced.

At this stage a complete separation of resistant and susceptible males was effected in a single selection by breeding, on one hand, from males which survived a topically applied dose of 32·0 μgm. DDT/gm. body-weight, and, on the other hand, from males which when tested later were killed by a dose of 11·2 μgm. DDT/gm. The two strains so obtained were designated *EY* (early, with resistance involving *Y*) and *ES* (early, susceptible).

Males and females of *ES* and females of *EY* constitute three similar and apparently homogeneous populations in respect to their tolerances to DDT, which lie between 2·0 and 11·2 μgm./gm. *EY* males are consistently about eight times as resistant, their tolerances ranging from 16·0 to 128·0 μgm./gm.

Strain *EY* has bred true without any applied pressure since the initial selection. All its males are specifically DDT-resistant and all its females non-resistant. It contrasts markedly with previously described strains of houseflies, for in these resistance to DDT has been found to be autosomally determined and not sex-linked or sex-limited[2–4]. A fuller account of this work will be published elsewhere.

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[1] Kerr, R. W., Venables, D. G., Roulston, W. J., and Schnitzerling, H. J., *Nature*, **180**, 1132 (1957).
[2] Milani, R., *Ric. Paras.*, **17**, 233 (1956); **18**, 43 (1957).
[3] Crow, J. F., *Ann. Rev. Ent.*, **2**, 227 (1957).
[4] Brown, A. W. A., World Health Organization Monograph Series No. 38 (1958).

BACTERIOLOGY

Intermediary Metabolism of Nitrobenzoic Acids by Bacteria

Ke *et al.*[1] have recently reported that the aerobic degradation of *o*-nitrobenzoic acid by a *Flavobacterium* involves the *o*-nitroso and *o*-hydroxylamino compounds. The corresponding amino-derivative, anthranilic acid, was not oxidized, their paper implying on the basis solely of manometric data that it was not a potential metabolite of *o*-nitrobenzoic acid although they found that cells grown on anthranilic acid could oxidize both it and salicylic acid. We wish to direct attention to the fact that although the aminobenzoates may not always be directly concerned in aerobic degradation of nitrobenzoic acids, they are nevertheless important intermediary metabolites.

We had previously recorded identical responses to those reported by Ke *et al.*, with *p*-nitrobenzoic acid and its derivatives, using cells of *Nocardia erythropolis* grown in the presence of *p*-nitrobenzoic acid, and with *o*-nitrobenzoic acid using cells of *N. opaca*, grown in a medium containing *o*-nitrobenzoic acid[2]. Although the aminobenzoic acid in both cases was not appreciably oxidized, it nevertheless accumulated in culture media. Work with cell-free extracts has shown that in addition to oxidation of the substrate, *N. erythropolis*, at least, carries out an appreciable, simultaneous reduction of the *para*-isomer to *p*-aminobenzoic acid by a pathway involving *p*-nitrosobenzoic and *p*-hydroxylaminobenzoic acids[3].

In contrast to Durham's earlier work[4,5] and the recent report of Ke *et al.*[1], no further oxidation of the aminobenzoates through the corresponding hydroxybenzoates has been found with our species of *Nocardia*. Cells of *N. opaca* grown on anthranilic acid failed to oxidize salicylate and growth of *N. erythropolis* was not maintained on *p*-aminobenzoic acid[6]. The use of desiccator-dried preparations of *N. opaca* showed that permeability factors were not responsible for absence of activity in that particular case.

The scheme of Ke *et al.*[1] whereby *o*-hydroxylaminobenzoic acid is metabolized to cellular material without passing through anthranilic acid is interesting in view of some results we obtained with *N. erythropolis*, which oxidizes *p*-nitrobenzoic acid through *p*-hydroxybenzoic acid to protocatechuic acid, also without the mediation of the amino-compound.

Table 1. PRODUCTION OF PROTOCATECHUIC ACID FROM *p*-NITROBENZOIC ACID AND ITS REDUCTION PRODUCTS BY *N. erythropolis*

Substrate	Protocatechuic acid produced		
	Exp. 1*	Exp. 2*	Exp. 3*
p-Nitrobenzoic acid	++++	++++	+++
p-Nitrosobenzoic acid	±	±	−
p-Hydroxylaminobenzoic acid	+++	+++	+
p-Aminobenzoic acid	−	−	−
None	−	−	−

−, ±, +, ++, etc., Intensity of protocatechuic acid spots estimated visually.

* Identical sheets were marked in the areas corresponding to protocatechuic acid, the areas cut out, eluted and a full spectrum read (210–320 mμ).

Experiment 1 was made with 24-hr. grown cells; experiment 2 with 48-hr. grown cells and experiment 3 with cells grown for 7 days, on *p*-nitrobenzoate.

Washed cell suspensions of *N. erythropolis* (grown on *p*-nitrobenzoate medium) in *tris* buffer with low aeration rates (see Cartwright and Cain[2] for full experimental details of this and all other methods used) produced appreciable amounts of protocatechuic acid from both *p*-nitro and *p*-hydroxylaminobenzoic acids, but much smaller amounts from *p*-nitrosobenzoic acid and none from the amino-compound. It was detected chromatographically in three solvent systems, identified spectrophotometrically (Table 1) and also isolated in pure form from a *p*-nitrobenzoate incubation mixture. The *p*-hydroxylaminobenzoic acid sample used was recrystallized several times from water prior to use and was shown to be quite free of nitro-compound by paper chromatography.

The results would suggest a pathway from *p*-hydroxylaminobenzoic acid to protocatechuic acid and terminal oxidation products lacking an intermediate amino-stage and support the results of Ke *et al.* with the *ortho*-isomer. Although *p*-nitro, *p*-nitroso and *p*-hydroxylaminobenzoic acids were oxidized at similar rates by cells grown on *p*-nitrobenzoic acid, the effects of inhibitors (particularly 1 m*M* nitrite) and other findings[2] still lead us to maintain that, with *Nocardia*, nitrite is the immediate nitrogenous product and that rapid reduction to ammonia is achieved by nitrite reductase activity. This reflects, as Ke *et al.*[1] have pointed out, the great diversity of pathways by which different micro-organisms may degrade the same substrate, but we must advocate the necessity for carrying out concomitant chemical studies with adaptation results of this kind. Our own results have shown that although *p*-aminobenzoic acid is not a direct intermediate in the primary aerobic, energy-producing pathway of *p*-nitrobenzoic acid degradation, it is eventually metabolized both in *p*-nitrobenzoate cultures and after incubation with washed cells of *N. erythropolis* grown on *p*-nitrobenzoate[3]. We have since identified further aminophenyl compounds produced in cultures from *p*-nitrobenzoate and from *o*-nitrobenzoate together with the corresponding hydroxynitrobenzoic acids (Table 2) and detected at least six other unidentified phenolic compounds from the former, none of which is oxidized by the organisms. Recent work (which will be reported in full elsewhere) on anthranilic acid metabolism by *N. opaca* confirms the multiplicity of compounds to which this aminobenzoate may be converted in cultures. This, we think, serves to emphasize the imprudence of eliminating a compound as a major metabolite merely from adaptation results based on manometric studies. If not involved in aerobic pathways, it may well be important in others.

Table 2. SOME AMINOPHENYL AND RELATED METABOLITES PRODUCED BY *Nocardia* SPP. FROM NITROBENZOIC ACIDS

Substrate	Species	Metabolites identified	First appears in cultures (days)
p-Nitrobenzoic acid	*Nocardia erythropolis*	*p*-aminobenzoic acid	1
		3-hydroxy-4-nitrobenzoic acid	3
		3-hydroxy-4-aminobenzoic acid	3
o-Nitrobenzoic acid	*N. opaca*	3-hydroxy-2-nitrobenzoic acid	3
		3-hydroxyanthranilic acid	3
		anthranilic acid	1
		o-aminophenol	3–4

These metabolites were found both in cultures of the two species and in incubation mixtures of washed cells of the respective species in 0·05 *M tris* or phosphate buffer, *p*H 7·2, with *p*- or *o*-nitrobenzoic acid at 0·01 *M*. Incubation temperature in both cases was 30°.

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1 Ke, Y-H., Lynn, L. G., and Durham, N. N., *J. Bacteriol.*, **77**, 593 (1959).
2 Cartwright, N. J., and Cain, R. B., *Biochem. J.*, **71**, 248 (1959).
3 Cartwright, N. J., and Cain, R. B., *Biochem. J.*, **73**, 305 (1959).
4 Durham, N. N., *J. Bacteriol.*, **72**, 333 (1956).
5 Durham, N. N., *Canad. J. Microbiol.*, **4**, 141 (1958).
6 Cain, R. B., *J. Gen. Microbiol.*, **19**, 1 (1958).

Intrastrain Transduction in *Proteus mirabilis*

A NUMBER of bacteriophage-mediated transduction systems have been described[1]. Intrastrain transduction was originally described for *Salmonella typhimurium* by Zinder and Lederberg[2] and recently Loutit has described a similar situation in *Pseudomonas aeruginosa*[3].

In an attempt to discover transducing systems in the genus *Proteus* for use in genetic transfer between *vulgaris* and *mirabilis* species, lysogeny in this genus was investigated[4]. Two of the temperate phages isolated[5] have been successfully employed in intrastrain transductions. The first was present in the supernatant of 10-day-old cultures of *Proteus mirabilis* strain 34[6] and formed turbid plaques on *Proteus mirabilis* strain 13; it is named phage 34/13. Phage 12/57 was obtained after ultra-violet irradiation[5] of *Proteus mirabilis* strain 12; it forms turbid plaques on *Proteus mirabilis* strain 57. The two transducing systems are analogous and as results are very similar only the one employing phage 12/57 will be described.

Streptomycin-resistant mutants (57/*S*) of the wild type 57 were obtained by plating about 10^{11} cells on a MacConkey-type agar[7] containing 1,000 μgm. streptomycin sulphate/ml. Colonies which arose were picked off and purified by re-streaking on a streptomycin medium.

Phage 12/57 does not produce clearing of broth cultures of 57 or 57/*S*, and in order to obtain high-titre lysates, recourse was taken to the modification of the confluent plaque method of Hershey *et al.*[8] described by Adams[9]. Successful experiments produced lysates of 57 or 57/*S* with plaque titres of 10^{10}/ml. as estimated by the agar layer method[10]. Lysates were centrifuged to clarity and rendered bacteriologically sterile by addition of chloroform.

Transduction experiments were conducted in a 37° C. water-bath and consisted of mixing 2 ml. of a 57/*S* lysate (about 2×10^{10} plaque-forming particles) with a deposit of about 6×10^8 wild type 57 cells obtained by centrifuging 1 ml. of an aerated overnight culture. The experiments were duplicated using a lysate of the wild type 57 of identical titre. No visible clearing occurs, and 15 min. after mixing, 0·1 ml. volumes were spread on a number of Difco *SS* agar plates. At 30-min. intervals 0·1 ml. volumes were spread on MacConkey agar containing 1,000 μgm. streptomycin sulphate/ml. After overnight incubation the *SS* plates were replicated[11] on to MacConkey agar containing 1,000 μgm. streptomycin sulphate/ml. An organism control (6×10^8 wild-type 57 in 2 ml. broth) and a lysate sterility control (2 ml. of the lysate) were similarly treated.

Replica plates of the *SS* agar in experiments using lysate from 57/*S* usually yielded about 100 colonies per plate, while those in experiments using the lysate from wild 57 were always sterile. Replica plates from organism controls were usually barren but occasionally showed 1 or 2 colonies per experiment. Lysate controls invariably confirmed their bacteriological sterility.

In experiments using lysates from 57/*S* the half-hourly direct platings on MacConkey agar containing 1,000 μgm. streptomycin sulphate/ml. first yielded colonies 2 hr. after mixing organisms and lysate. The corresponding plates in experiments using lysates of wild 57, and in the organism and lysate controls, never showed growth.

The transducing activity of 57/*S* lysates is not impaired by treatment with deoxyribonuclease (40 μgm. per ml. for 2 hr. at 37° C.) but is totally inhibited by phage 12/57 rabbit antiserum, which reduces the plaque titre from 10^{10} to 10^4/ml. The addition of a similar volume of normal rabbit serum to the lysate has no such effect.

Results outlined place this experiment in the class of bacteriophage-transmitted intrastrain genetic exchange.

The streptomycin resistance marker has been the only one employed to date. The high mutation-rate of wild 57 and wild 13 to sodium azide resistance precludes the use of this property as a marker. No biochemical or nutritional mutants have yet been recovered.

The transduction frequency per phage particle is about 10^{-7}, and is comparable with rates encountered in common transduction systems[12]. There appears to be a delay in phenotypic expression of transduced streptomycin resistance. This delay has also been encountered with the transduction of streptomycin resistance in the *Salmonella typhimurium* system[13]. As in the latter system no abortive transductions have been detected.

Study of the transduced organisms has proved that the streptomycin resistance is a stable characteristic. These organisms do not differ biochemically from the wild type. None of the colonies tested to date adsorbs phage present in either 57 or 57/*S* lysates. Mutants of wild 57 resistant to phage present in lysates of 57 are not resistant to streptomycin. The mechanism by which the streptomycin-resistant transductants of 57 become resistant to phage is being investigated.

This work was aided by grants from the South African Council of Scientific and Industrial Research.

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[1] Stocker, B. A. D., in "Recent Progress in Microbiology", ed. by Tunevall, G., 31 (Blackwell Scientific Publications, Oxford, 1959).
[2] Zinder, N. D., and Lederberg, J., *J. Bact.*, **64**, 679 (1952).
[3] Loutit, J. S., *J. Gen. Microbiol.*, **18**, 315 (1958).
[4] Coetzee, J. N., and Sacks, T. G., *Nature*, **184**, 1340 (1959).
[5] Coetzee, J. N., and Sacks, T. G., *S. Afr. J. Lab. Clin. Med.* (in the press).
[6] Coetzee, J. N., *S. Afr. J. Lab. Clin. Med.*, **4**, 147 (1958).
[7] Coetzee, J. N., *S. Afr. J. Lab. Clin. Med.*, **5**, 16 (1959).
[8] Hershey, A. D., Kalmanson, G., and Bromfenbrenner, J., *J. Immunol.*, **46**, 267 (1943).
[9] Adams, M. H., in "Methods in Medical Research", ed. by Comroe, J. H., **2**, 12 (The Year Book Publishers, Chicago, 1956).
[10] Adams, M. H., in "Methods in Medical Research", ed. by Comroe, J. H., **2**, 7 (The Year Book Publishers, Chicago, 1956).
[11] Lederberg, J., and Lederberg, E. M., *J. Bact.*, **63**, 399 (1952).
[12] Bertani, G., in "Advances in Virus Research", ed. by Smith, K. M., and Laufer, M. A., **5**, 182 (Academic Press, New York, 1958).
[13] Watanabe, T., and Watanabe, M., *J. Gen. Microbiol.*, **21**, 16 (1959).

Characteristics of *Thiobacillus thioparus*

During investigations of the bacterial decomposition of thiosulphate and thiocyanate in effluents from gasworks, it became necessary to identify the organisms responsible. These were *Thiobacilli*, and one in particular, organism *G*, appeared identical with *T. thioparus* as described in Bergey[1]. To confirm this, its behaviour was compared with various *Thiobacilli* from the National Collection of Industrial Bacteria. As a result of this comparison it was discovered that the type species, *T. thioparus* (NCIB 8370), did not correspond with Bergey's classification on two important points: it grew on nutrient agar, and it grew anaerobically on thiosulphate containing potassium nitrate. To check this a further specimen of the organism was obtained, and although this was again a pure culture it showed precisely the same characteristics.

After discussion with a member of the staff of the National Chemical Laboratory, it was concluded that due to difficulties associated with the culture originally given to the Collection, NCIB 8370 was probably not *T. thioparus*.

The matter is of some importance, as strain NCIB 8370 was used by De Kruyff *et al.*[2] to show that *T. thioparus* and *T. thiocyanoxidans* are identical; as it seems not to be *T. thioparus* their conclusions would appear to be invalid.

We wish to thank the Joint Research Committee of the Gas Council and the University of Leeds for permission to publish this communication.

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[1] Bergey's "Manual of Determinative Bacteriology", 7th ed. (Baillière, Tindall and Cox, 1957).
[2] De Kruyff, C. D., van der Walt, J. P., and Schwartz, H. M. "Antonie van Leeuwenhoek", **23**, 305 (1957).

FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, March 21

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. E. K. Woodford and Mr. L. Kasasian: "Some New Herbicides and Developments in Chemical Weed Control 1958–60".

MANCHESTER LITERARY AND PHILOSOPHICAL SOCIETY (in the Reynolds Hall, Manchester College of Science and Technology, Sackville Street, Manchester), at 5.45 p.m.—Mr. C. E. Young: "The Growing Importance of Metallo-Organic Compounds" (Presidential Address).

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "The Computer in Production".

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (at Savoy Place, London, W.C.2), at 6.30 p.m.—Dr. F. A. Benson: "Developments in Lighting".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Martin W. Holdgate: "Islands of Western Patagonia".

Tuesday, March 22

BRITISH MUSEUM (NATURAL HISTORY) (in the Lecture Hall, Cromwell Road, London, S.W.7), at 4.30 p.m.—Prof. Charles G. Sibley (Cornell University): "Genes, Proteins and Evolution—The Phylogeny of Birds as indicated by Molecular Structure".*

INSTITUTE OF PHYSICS, ELECTRONICS GROUP (at 47 Belgrave Square London, S.W.1), at 5.30 p.m.—Annual General Meeting, followed by Mr. P. O. Hawkins: "Neutron Sources".

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. R. L. Brown and Mr. J. C. Richardson: "Profile of Flow of Granules Through Apertures".

Tuesday, March 22—Thursday, March 24

ROYAL INSTITUTION OF NAVAL ARCHITECTS (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 10.15 a.m. daily—Spring Meeting.

Wednesday, March 23

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. H. R. Gregory: "A New Method of Briquetting Coal without a Binder".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. G. Millington: "The Challenge of the Propagation Medium to the Radio Engineer".

ROYAL PHOTOGRAPHIC SOCIETY, SCIENTIFIC AND TECHNICAL GROUP (joint meeting with the COLOUR GROUP of the PHYSICAL SOCIETY, in the Lecture Theatre of the Science Museum, South Kensington, London, S.W.7), at 5.30 p.m.—Mr. M. H. Wilson: "Two-colour Projection Phenomena".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the Courtauld Hall, Queen Elizabeth College, Campden Hill Road, London, W.8), at 6 p.m.—Scientific Papers (Eighth Young Members' Meeting).

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL AND SOCIAL PSYCHOLOGY SECTIONS (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Dr. R. Money-Kyrle and Dr. Marie Jahoda: "Prejudice".

Thursday, March 24

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Meeting for the Election of Fellows. Mr. A. G. Swallow and Miss Mary R. Truter: "The Stereochemistry of β-Diketo Complexes with Trimethylplatinum IV. I: The Crystal Structure of Trimethyl 4 : 6-Dioxononylplatinum"; Mr. A. C. Hazell and Miss Mary R. Truter: "The Stereochemistry of β-Diketo Complexes with Trimethylplatinum IV. II: The Crystal Structure of Ethyl (Trimethylplatini-)acetoacetate".

INSTITUTION OF MECHANICAL ENGINEERS, APPLIED MECHANICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "A Replica Technique for Measuring Static Strains".

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. R. R. Jamison and Mr. R. J. Lane: "Engines for Supersonic Air Liners".

Thursday, March 24—Friday, March 25

NATIONAL ASSOCIATION FOR MENTAL HEALTH (at Church House, Westminster, London, S.W.1)—Annual Conference—"Mental Health at Home and Abroad".

Friday, March 25

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. D. Perkins: "Aerodynamics of Man Powered Flight, with special reference to Inflatable Wings".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Ronald King: "Tyndall, The Teacher".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

COWBURN RESEARCH FELLOW, to undertake research on certain aspects of immunology in relation to cancer—The Secretary, King's College Hospital Medical School, Denmark Hill, London, S.E.5 (April 2).

ASSISTANT EXPERIMENTAL OFFICER (with a science degree preferably including bacteriology and chemistry, and some experience in bacteriological work) IN THE BACTERIOLOGY DEPARTMENT—The Secretary, Hannah Dairy Research Institute, Kirkhill, Ayr (April 4).

ASSISTANT LECTURER IN PHYSIOLOGY OR PHARMACOLOGY—The Registrar, The University, Nottingham (April 4).

SENIOR LECTURER IN APPLIED THERMODYNAMICS; a SENIOR LECTURER IN FLUID MECHANICS; a SENIOR LECTURER IN MECHANICS OF MACHINES; a SENIOR LECTURER IN STRENGTHS OF MATERIALS; a SENIOR LECTURER IN PRODUCTION TECHNOLOGY; and a LECTURER IN MECHANICAL ENGINEERING—The Registrar, Royal Technical College, Salford 5, Lancs (April 4).

ASSISTANT (preferably with special interests in the biochemistry or micro-organisms, or in physical biochemistry) IN BIOCHEMISTRY—The Secretary of University Court, The University, Glasgow (April 8).

ASSISTANT (with a degree in agriculture, horticulture or economics) TO THE HORTICULTURAL LIAISON OFFICER IN THE DEPARTMENT OF AGRICULTURAL ECONOMICS—The Registrar, The University, Nottingham (April 9).

ASSISTANT LECTURER (preferably with a knowledge of some branch of comparative physiology, especially neurophysiology) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, University College of Wales, Aberystwyth (April 9).

LECTURER (with a good honours degree in electrical engineering or the equivalent, and ability to participate effectively in research work in this field) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, with special reference to the transmission and distribution of electric power—Prof. A. Tustin, Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7 (April 9).

SENIOR LECTURER IN VIROLOGY, to be responsible for the organization of virology and for teaching in the Department of Pathology—The Secretary, Medical College of St. Bartholomew's Hospital, West Smithfield, London, E.C.1 (April 9).

LECTURER IN AGRICULTURAL CHEMISTRY—The Secretary of University Court, The University, Glasgow (April 11).

LECTURER IN ASTRONOMY in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews, Fife (April 16).

UNIVERSITY LECTURERS (2) (one with special reference to mycology and plant pathology and the other to ecology) IN BOTANY—Dr. C. B. Goodhart, Museum of Zoology, The University, Downing Street, Cambridge (April 18).

CANCER RESEARCH FELLOW (with training in cytology), for duties mainly in connexion with the study of human chromosomes in patients with leukæmia and associated diseases—The Registrar, The University, Liverpool, quoting Ref. CV/N (April 19).

CHAIR OF ZOOLOGY in the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 20).

SENIOR LECTURER or LECTURER IN ANATOMY; and a SENIOR LECTURER or LECTURER IN PHYSIOLOGY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 20).

LECTURER/SENIOR LECTURER (with either travel or field experience in Greece and Italy or the Near East) IN CLASSICAL or NEAR EASTERN ARCHÆOLOGY, at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 22).

ASSISTANT LECTURER (with special qualifications in physical chemistry) IN CHEMISTRY—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1 (April 23).

ASSISTANT LECTURER or LECTURER (preferably with a special interest in some group of fossils common to the lower Palæozoic rocks) IN PALÆONTOLOGY—The Registrar, University College of Wales, Aberystwyth (April 25).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY OR PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

LECTURER (graduate in science) IN MICROBIOLOGY—The Secretary, The Queen's University, Belfast (April 30).

LECTURER IN INORGANIC CHEMISTRY—The Registrar, The University, Manchester 13 (April 30).

RESEARCH ASSISTANT (with an honours degree in mathematics or a related subject, and preferably experience in the use of digital computers) IN THE COMPUTING LABORATORY—The Secretary of University Court, The University, Glasgow (April 30).

SENIOR LECTURER IN PHYSICS; and a LECTURER IN PHYSICS at the University of Natal, Pietermaritzburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, April 30).

TURNER AND NEWELL RESEARCH FELLOW IN ENGINEERING, INORGANIC CHEMISTRY OR PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 30).

UNIVERSITY STUDENTS AND FELLOWS, for experimental or theoretical research in nuclear and elementary particle physics—The Professor of Natural Philosophy, The University, Glasgow (April 30).

CHAIR OF CHEMISTRY in the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 30).

BIOCHEMIST (interested in biological assay work) FOR ENDOCRINOLOGY LABORATORY, working in connexion with cancer research—The House Governor, Royal Marsden Hospital, Fulham Road, London, S.W.3.

BIOCHEMIST (preferably with previous hospital experience) IN THE PATHOLOGICAL LABORATORY—The Secretary, Highlands General Hospital, Winchmore Hill, London, N.21.

CHEMIST or AGRICULTURAL CHEMIST (with an honours degree in science or equivalent qualification, and preferably postgraduate research experience to Ph.D. level, and a knowledge of agriculture), to undertake research on the quality of protein in animal feeding stuffs for non-ruminants—The Secretary, The Rowett Research Institute, Bucksburn, Aberdeen.

CYTOLOGIST (Scientific or Senior Scientific Officer grade) AT THE WELSH PLANT BREEDING STATION—The Registrar, University College of Wales, Aberystwyth.

DARWIN RESEARCH FELLOW (with suitable qualifications in science or medicine), for research in the field of human population genetics with special reference to the range of human variability—The Eugenics Society, 69 Eccleston Square, London, S.W.1.

EDITOR (chemist, with editorial experience) OF *Chemistry and Industry*—The General Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1, marking envelope "Personal".

LECTURER (with good academic qualifications and either experience of, or special interest in, nuclear physics) IN PHYSICS, at Bournemouth Municipal College of Technology and Commerce, to help with the development of courses in nuclear and reactor physics and teach physics to Grad.Inst.P., H.N.C. applied physics and B.Sc. (General) levels—Chief Education Officer, Town Hall, Bournemouth.

MASTER TO TEACH CHEMISTRY throughout the School—The Headmaster, Malvern College, Malvern.

MICROBIOLOGIST (with research experience), for work on radiation-induced mutations in microbial populations—The Administrator, Radiobiological Research Unit, Medical Research Council, Harwell, Berks.

PHYSICISTS (with doctorate degree, and preferably a fair knowledge of French)—Le Directeur, Departement de Physique, Université de Montréal, Canada.

RESEARCH ASSISTANT (graduate), to join a group working on problems of cancer research in the border-line fields of biology and chemistry—The Administrative Officer, Mount Vernon Hospital, Northwood, Middlesex.

RESEARCH POST IN THE RADIOASTRONOMY GROUP, to participate in an expanding programme of research in upper atmosphere physics, including investigations of meteors, auroræ and solar-terrestrial relations—Dr. T. R. Kaiser, Department of Physics, The University, Sheffield 10.

TECHNICIAN or JUNIOR TECHNICIAN (interested in biochemistry), for biochemical and biological assay work in the ENDOCRINOLOGY LABORATORY—The House Governor, Royal Marsden Hospital, Fulham Road, London, S.W.3.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Registrar General's Statistical Review of England and Wales for the year 1957. Part 3: Commentary. Pp. xiv+299. (London: H.M. Stationery Office, 1959.) 16*s.* 6*d.* net. [3012

Time Measurement in Dynamic Geometry. By S. Ryzewski. Pp. 16. (Southampton: S. Ryzewski, 15 Landguard Road, 1959.) [3012

Africa: Social Change and Mental Health. (Report of a Panel Discussion conducted in Conference Room No. 1, United Nations, New York, March 23, 1959, by the World Federation for Mental Health). Pp. 40. (London: World Federation for Mental Health, 1959. Distributed by H. K. Lewis and Co., Ltd.) 3*s.* 6*d.* net. [3012

Privy Council. Medical Research Council Memorandum No. 37: Sewage Contamination of Bathing Beaches in England and Wales. Pp. iv+24. (London: H.M. Stationery Office, 1959.) 2*s.* 6*d.* net. [3012

National Museum of Wales. Fifty-second Annual Report, 1958–59. Pp. 79+4 plates. (Cardiff: National Museum of Wales, 1959.) [61

Review of Coal Tar Technology, January–June, 1959. (Vol. 11, Part 1.) Pp. ii+83. (Gomersal, Leeds: Coal Tar Research Association, 1959.) 10*s.* [61

Institute of Personnel Management. Occasional Papers, No. 15: Working Wives—a Survey of Facts and Opinions concerning the Gainful Employment of Married Women in Britain. Carried out in Co-operation with Mass Observation, Ltd. by Viola Klein. Pp. 63. (London: Institute of Personnel Management, 1960.) 7*s.* 6*d.* [61

Iodine Tests and Reagents in Chemical Analysis. Pp. i+52. (London: Chilean Iodine Educational Bureau, 1959.) [61

Bulletin of the British Museum (Natural History). Historical Series. Vol. 2, No. 2: Darwin's Notebooks on Transmutation of Species. Part 1: First Notebook (July 1837–February 1838). Edited with an Introduction and Notes by Sir Gavin de Beer. Pp. 23–73. (London: British Museum (Natural History), 1960.) 20*s.* [61

Annual Report of the Challenger Society, 3, No. XI. Pp. 38. (Wormley, Godalming: Challenger Society, c/o National Institute of Oceanography, R. I. Currie, Hon. Secretary, 1959.) [61

Annual Report of the National Oceanographic Council, 1 April 1958–31 March 1959. Pp. v+41. (Cambridge: At the University Press, 1959.) 5*s.* net. [61

British Broadcasting Corporation. The Public and the Programmes: an Audience Research Report on Listeners and Viewers, the time they devote to listening and viewing, the Services they patronize, their Selectiveness and their Tastes. Pp. 71. (London: British Broadcasting Corporation, 1959.) [61

Building Research Station Digest No. 129: Cranes for Building. Pp. 6. (London: H.M. Stationery Office, 1959.) 4*d.* [61

Ministry of Agriculture, Fisheries and Food. Bulletin No. 77: Tomatoes—Cultivation, Diseases and Pests. Fifth edition. Pp. v+74+8 plates. (London: H.M. Stationery Office, 1959.) 5*s.* 6*d.* net. [61

Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 1, No. 1: Radiocarbon Dating System Using Scintillation Techniques. By C. F. G. Delaney and I. R. McAuley. Pp. 1–20. (Dublin: Royal Dublin Society, 1959.) 4*s.* [61

Economic Proceedings of the Royal Dublin Society. Vol. 4, No. 7: Observations for Eleven Years on a Liming Trial. By P. Bergin. Pp. 119–136. 4*s.* Vol. 4, No. 8: Chemical and Growth Gradients of *Laminaria cloustoni* Edm. (*L. Hyperborea* Fosl.) By W. A. P. Black, W. D. Richardson and F. T. Walker. Pp. 137–149. 3*s.* (Dublin: Royal Dublin Society, 1959.) [61

Ciba (A.R.L.), Limited. Technical Notes, No. 204 (December 1959): 'Araldite' in Slip Ring Assemblies. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1959.) [61

United Kingdom Atomic Energy Authority: Research Group. Report (AERE—R3159): Heat Transfer to Two Phase Gas-Liquid Systems. Part 1: Steam-Water Mixtures in the Liquid-Dispersed Region in an Annulus. By J. A. R. Bennett, J. G. Collier, H. R. C. Pratt and J. D. Thornton. Pp. iv+46+13 figures. Report (AERE—R3195): Pressure-Drop Data for the Vertical Flow of Air-Water Mixtures in the Climbing-Film and Liquid-Dispersed Regions. By J. A. R. Bennett and J. D. Thornton. Pp. ii+22+6 figures. (Harwell: Chemical Engineering Division, Atomic Energy Research Establishment, 1959.) [61

Horniman Museum and Library. A Handbook to the Collections Illustrating a Survey of the Animal Kingdom. Third edition. Pp. 92+4 plates. (London: London County Council, 1959.) 2*s.* 6*d.* [61

Northern Ireland Development Council. Freight Services to and from Northern Ireland. Second edition. Pp. 1+42+6 plates. (London: Northern Ireland Development Council, 1960.) [61

Department of Scientific and Industrial Research. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1958, with the Report of the Geological Survey Board. Pp. iii+77. (London: H.M. Stationery Office, 1959.) 5*s.* net. [61

The National Union of Teachers. University Entrance—The Basic Facts. Pp. 48. (London: National Union of Teachers, 1960.) 3*s.* 6*d.* [61

British Medical Bulletin, Vol. 16, No. 1 (January 1960): Antibiotics in Medicine. Pp. 1–88+xvii. (London: The British Council, 1960.) 20*s.* [61

International Commission for Uniform Methods of Sugar Analysis. Report of the Proceedings of the Twelfth Session held at the National Bureau of Standards and at the Shoreham Hotel, Washington, D.C., U.S.A., from June 2nd to June 6th, 1958. Pp. vii+130. (Keston, Kent: International Commission for Uniform Methods of Sugar Analysis, Tate and Lyle Research Laboratories; New York: Sugar Research Foundation, Inc., 52 Wall Street, 1959.) 25*s.*; 3.50 dollars. [61

Colour Breeding of Syrian Hamsters. By Roy Robinson. Pp. 16. (Ealing, London: Robin Bibliographies, St. Stephens Road Nursery, 1960.) 3*s.* 6*d.* [121

Other Countries

State of Singapore. Fisheries Bulletin No. 3: Fisheries Survey Report No. 2—The Fishing Gear of the State of Singapore. By T. W. Burdon. Pp. vi+166+10 plates. (Singapore: Government Publications Bureau, 1959.) 2.50 Malayan dollars. [3012

Institut pour la Recherche Scientifique en Afrique Centrale. Onzième Rapport Annuel, 1958. Pp. 375. (Bruxelles: Institut pour la Recherche Scientifique en Afrique Centrale, 1959.) [3012

Colony of Mauritius. Meteorological Observations and Climatological Summaries, 1958. Part 7: July. Pp. 14. Part 8: August. Pp. 14. Part 9: September. Pp. 14. Part 10: October. Pp. 14. Part 11: November. Pp. 14. Part 12: December. Pp. 25. (Port Louis: Government Printer, 1959.) [3012

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 51: Surficial Geology of Southern District of Keewatin and the Keewatin Ice Divide, Northwest Territories. By Hulbert A. Lee. Pp. vi+42 (13 plates). 50 cents. Memoir No. 298: Coalfields, West Half Cumberland County, Nova Scotia. Pp. vi+89. 75 cents. (Ottawa: Queen's Printer, 1959.) [61

La Science en Pologne Populaire, 1944–1959. Par Tadaesz Kotarbinski. (Rapport présenté à l'Assemblée Générale de l'Académie Polonaise des Sciences le 26 juin 1959.) Pp. 45. (Varsovie: Editions Scientifiques Polonaises, 1959.) [61

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 7: Pantograph Adjustment. By G. C. Tewinkel. Pp. ii+3. 5 cents. Technical Bulletin No. 10: Vertical Adjustment of Instrument Aerotriangulation by Computational Methods. By William D. Harris. Pp. ii+3. 10 cents. (Washington, D.C.: Government Printing Office, 1959.) [61

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University of Khartoum: Natural History Museum. Annual Report for period 1st July, 1956 to 30th June, 1958. Pp. 19. (Khartoum: University of Khartoum: Sudan Natural History Museum, 1959.) [61

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IN THE SERVICE OF YOUTH

THE second* of two recently published reports concerned with the way in which the State should assist in the service of youth has met with much more sympathetic public response than the first†. The Committee was set up by the Minister of Education just over a year ago, under the chairmanship of the Countess of Albemarle, and has completed its inquiry with exemplary promptness. Invited to "review the contribution which the Youth Service of England and Wales can make in assisting young people to play their part in the life of the community, in the light of changing social and industrial conditions and of current trends in other branches of the education service; and to advise according to what priorities best value can be obtained for the money spent", the Committee quickly realized that it had been convened none too soon and that speedy action would be required if the Youth Service were to flourish. It is not surprising to learn from the Report that the Committee was always aware that it was working against time and that the recommendations made should be regarded as in the nature of a blood transfusion. Side by side with the Committee's proposals for immediate adoption come proposals which would ensure sustained and continued nourishment if the blood transfusion succeeds in restoring the health of a sickly and dispirited child.

The main factor contributing to the sense of urgency which governed the Committee's inquiry is, of course, the 'bulge'. For every five young people between the ages of fifteen and twenty in Britain to-day there will be, in 1964, six young people. Although the increase will not be spread evenly across the country as a whole, all areas and each kind of area will show some increase; in some new towns the increase may be as much as five-fold. Between 1964 and 1970 the total number will decline gradually, but will still remain substantially higher than that of to-day. From 1970 there is again the probability of a gradual increase. The inadequate facilities which are available in the Youth Service to-day are quantitatively insufficient to meet demands which are already upon us. Measures taken to meet the impact of the 'bulge' in the schools and technical colleges and the universities have not been matched with similar provision to prepare the Youth Service for the needs of the increased population. The Committee quickly recognized that unless action is taken at once the 'bulge' generations will leave school only to find a Youth Service inadequate to cope even with its earlier responsibilities.

The effects of the 'bulge' will be reinforced by the cessation of National Service. During the past few years military commitments have kept about 200,000 young men between the ages of eighteen and twenty out of civilian life. Its gradual abandonment during the next two or three years will leave this number of young men available for civilian employment and leisure pursuits. Despite sincere beliefs that National Service has had a wholly unsatisfactory effect upon the lives and characters of many young men in leading to excessive drinking and sexual promiscuity, it is generally accepted that it has provided the majority of those serving with challenge and adventure suitable to their age and needs. The nation, and the young men themselves, will be the poorer unless the Youth Service can provide, in relevant civilian terms, opportunities to match those which come through National Service. The cessation of National Service and the arrival of the 'bulge' will make the Youth Service responsible for about a million more young people in 1964 than it had to cater for in 1958.

A third factor which strengthened the Albemarle Committee's belief that the Youth Service needed immediate support is the unfortunate emergence of a new climate of crime and delinquency. Without wishing to exaggerate the incidence of delinquency, which still involves in any one year only about 2 per cent of boys aged fourteen and less than twenty-one and only 0·2 per cent of girls of the same age group, it is still the fact that, of the total number of persons found guilty of indictable offences in 1957, about 50 per cent were less than twenty-one, and 30 per cent were aged 14–20 inclusive. Whether the figures are large or small in relation to the total population, the crime problem is largely a youth problem, and one in which the public can reasonably expect the Youth Service to play a considerable part in its solution.

To meet the challenge of the 'bulge' and the opportunities provided by the cessation of National Service, as well as to serve the nation in helping to combat the incidence of delinquency among young people, demands a Youth Service made up of virile and enthusiastic men and women dedicated to their calling. This was scarcely the position which confronted the members of the Albemarle Committee during their investigations. Instead, the Committee found leadership suffering from shortage of money and lack of encouragement. Leaders, feeling unsupported and unappreciated, have looked for some sign that their work is nationally recognized as important. They find it neither in official expressions of policy nor in the rewards of a salary scale for those who are full-time which would put the work on a level with cognate professions. They seem to themselves to be in danger of becoming cut off from the march of social and educational advance, and it is neither surprising that the total number of full-time youth leaders is declining nor that full-time posts are failing to attract applicants with the desired qualities.

* Ministry of Education. The Youth Service in England and Wales: Report of the Committee Appointed by the Minister of Education in November, 1958. Pp. iv+136. (Cmnd. 929.) (London: H.M. Stationery Office, 1960.) 6*s.* net.

† Ministry of Education. 15 to 18: A Report of the Central Advisory Council for Education (England). Vol. 1: Report. Pp. xxxi+519. (London: H.M. Stationery Office, 1959.) 12*s.* 6*d.* See also *Nature*, February 6, p. 341.

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The Registrar General's Statistical Review of England and Wales for the year 1957. Part 3: Commentary. Pp. xiv+299. (London: H.M. Stationery Office, 1959.) 16*s.* 6*d.* net. [3012

Time Measurement in Dynamic Geometry. By S. Ryzewski. Pp. 16. (Southampton: S. Ryzewski, 15 Landguard Road, 1959.) [3012

Africa: Social Change and Mental Health. (Report of a Panel Discussion conducted in Conference Room No. 1, United Nations, New York, March 23, 1959, by the World Federation for Mental Health). Pp. 40. (London: World Federation for Mental Health, 1959. Distributed by H. K. Lewis and Co., Ltd.) 3*s.* 6*d.* net. [3012

Privy Council. Medical Research Council Memorandum No. 37: Sewage Contamination of Bathing Beaches in England and Wales. Pp. iv+24. (London: H.M. Stationery Office, 1959.) 2*s.* 6*d.* net. [3012

National Museum of Wales. Fifty-second Annual Report, 1958–59. Pp. 79+4 plates. (Cardiff: National Museum of Wales, 1959.) [61

Review of Coal Tar Technology, January–June, 1959. (Vol. 11, Part 1.) Pp. ii+83. (Gomersal, Leeds: Coal Tar Research Association, 1959.) 10*s.* [61

Institute of Personnel Management. Occasional Papers, No. 15: Working Wives—a Survey of Facts and Opinions concerning the Gainful Employment of Married Women in Britain. Carried out in Co-operation with Mass Observation, Ltd. by Viola Klein. Pp. 63. (London: Institute of Personnel Management, 1960.) 7*s.* 6*d.* [61

Iodine Tests and Reagents in Chemical Analysis. Pp. i+52. (London: Chilean Iodine Educational Bureau, 1959.) [61

Bulletin of the British Museum (Natural History). Historical Series. Vol. 2, No. 2: Darwin's Notebooks on Transmutation of Species. Part 1: First Notebook (July 1837–February 1838). Edited with an Introduction and Notes by Sir Gavin de Beer. Pp. 23–73. (London: British Museum (Natural History), 1960.) 20*s.* [61

Annual Report of the Challenger Society, 3, No. XI. Pp. 38. (Wormley, Godalming: Challenger Society, c/o National Institute of Oceanography, R. I. Currie, Hon. Secretary, 1959.) [61

Annual Report of the National Oceanographic Council, 1 April 1958–31 March 1959. Pp. v+41. (Cambridge: At the University Press, 1959.) 5*s.* net. [61

British Broadcasting Corporation. The Public and the Programmes: an Audience Research Report on Listeners and Viewers, the time they devote to listening and viewing, the Services they patronize, their Selectiveness and their Tastes. Pp. 71. (London: British Broadcasting Corporation, 1959.) [61

Building Research Station Digest No. 129: Cranes for Building. Pp. 6. (London: H.M. Stationery Office, 1959.) 4*d.* [61

Ministry of Agriculture, Fisheries and Food. Bulletin No. 77: Tomatoes—Cultivation, Diseases and Pests. Fifth edition. Pp. v+74+8 plates. (London: H.M. Stationery Office, 1959.) 5*s.* 6*d.* net. [61

Scientific Proceedings of the Royal Dublin Society. Series A, Vol. 1, No. 1: Radiocarbon Dating System Using Scintillation Techniques. By C. F. G. Delaney and I. R. McAuley. Pp. 1–20. (Dublin: Royal Dublin Society, 1959.) 4*s.* [61

Economic Proceedings of the Royal Dublin Society. Vol. 4, No. 7: Observations for Eleven Years on a Liming Trial. By P. Bergin. Pp. 119–136. 4*s.* Vol. 4, No. 8: Chemical and Growth Gradients of *Laminaria cloustoni* Edm. (*L. Hyperborea* Fosl.) By W. A. P. Black, W. D. Richardson and F. T. Walker. Pp. 137–149. 3*s.* (Dublin: Royal Dublin Society, 1959.) [61

Ciba (A.R.L.), Limited. Technical Notes, No. 204 (December 1959): 'Araldite' in Slip Ring Assemblies. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1959.) [61

United Kingdom Atomic Energy Authority: Research Group. Report (AERE—R3159): Heat Transfer to Two Phase Gas-Liquid Systems. Part 1: Steam-Water Mixtures in the Liquid-Dispersed Region in an Annulus. By J. A. R. Bennett, J. G. Collier, H. R. C. Pratt and J. D. Thornton. Pp. iv+46+13 figures. Report (AERE—R3195): Pressure-Drop Data for the Vertical Flow of Air-Water Mixtures in the Climbing-Film and Liquid-Dispersed Regions. By J. A. R. Bennett and J. D. Thornton. Pp. ii+22+6 figures. (Harwell: Chemical Engineering Division, Atomic Energy Research Establishment, 1959.) [61

Horniman Museum and Library. A Handbook to the Collections Illustrating a Survey of the Animal Kingdom. Third edition. Pp. 92+4 plates. (London: London County Council, 1959.) 2*s.* 6*d.* [61

Northern Ireland Development Council. Freight Services to and from Northern Ireland. Second edition. Pp. i+42+6 plates. (London: Northern Ireland Development Council, 1960.) [61

Department of Scientific and Industrial Research. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1958, with the Report of the Geological Survey Board. Pp. iii+77. (London: H.M. Stationery Office, 1959.) 5*s.* net. [61

The National Union of Teachers. University Entrance—The Basic Facts. Pp. 48. (London: National Union of Teachers, 1960.) 3*s.* 6*d.* [61

British Medical Bulletin, Vol. 16, No. 1 (January 1960): Antibiotics in Medicine. Pp. 1–88+xvii. (London: The British Council, 1960.) 20*s.* [61

International Commission for Uniform Methods of Sugar Analysis. Report of the Proceedings of the Twelfth Session held at the National Bureau of Standards and at the Shoreham Hotel, Washington, D.C., U.S.A., from June 2nd to June 6th, 1958. Pp. vii+130. (Keston, Kent: International Commission for Uniform Methods of Sugar Analysis, Tate and Lyle Research Laboratories; New York: Sugar Research Foundation, Inc., 52 Wall Street, 1959.) 25*s.*; 3.50 dollars. [61

Colour Breeding of Syrian Hamsters. By Roy Robinson. Pp. 16. (Ealing, London: Robin Bibliographies, St. Stephens Road Nursery, 1960.) 3*s.* 6*d.* [121

Other Countries

State of Singapore. Fisheries Bulletin No. 3: Fisheries Survey Report No. 2—The Fishing Gear of the State of Singapore. By T. W. Burdon. Pp. vi+166+10 plates. (Singapore: Government Publications Bureau, 1959.) 2.50 Malayan dollars. [3012

Institut pour la Recherche Scientifique en Afrique Centrale. Onzième Rapport Annuel, 1958. Pp. 375. (Bruxelles: Institut pour la Recherche Scientifique en Afrique Centrale, 1959.) [3012

Colony of Mauritius. Meteorological Observations and Climatological Summaries, 1958. Part 7: July. Pp. 14. Part 8: August. Pp. 14. Part 9: September. Pp. 14. Part 10: October. Pp. 14. Part 11: November. Pp. 14. Part 12: December. Pp. 25. (Port Louis: Government Printer, 1959.) [3012

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin No. 51: Surficial Geology of Southern District of Keewatin and the Keewatin Ice Divide, Northwest Territories. By Hulbert A. Lee. Pp. vi+42 (13 plates). 50 cents. Memoir No. 298: Coalfields, West Half Cumberland County, Nova Scotia. Pp. vi+89. 75 cents. (Ottawa: Queen's Printer, 1959.) [61

La Science en Pologne Populaire, 1944–1959. Par Tadeusz Kotarbinski. (Rapport présenté à l'Assemblée Générale de l'Académie Polonaise des Sciences le 26 juin 1959.) Pp. 45. (Varsovie: Editions Scientifiques Polonaises, 1959.) [61

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